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Original Article

Effects of miR-129-5p on inflammation and nucleus pulposus cell apoptosis in rats with intervertebral disc degeneration through JNK signaling pathway

Chen Li[1], Zhiwen Sun[2], Hongjun Lou[3], Yenong Sun[4], Chengyuan Li[5], Xi Gao[1,*]

[1] Fourth Department of Bone Injury Diseases, First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China
[2] First Department of Digestive Diseases, First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China
[3] Department of Clinical Laboratory, First Affiliated Hospital of Heilongjiang University of Chinese Medicine, Harbin, China
[4] Department of Clinical Laboratory, Daqing Hospital of Traditional Chinese Medicine, Daqing, China
[5] School of Graduate, Heilongjiang University of Chinese Medicine, Harbin, China

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Abstract

This study aimed to explore the effects of miR-129-5p on inflammation and nucleus pulposus (NP) cell apoptosis in rats with intervertebral disc degeneration (IVDD) through the c-Jun N-terminal kinase (JNK) signaling pathway. A total of 20 rats were randomly divided into control group (n=10) or IVDD group (n=10). The mRNA expressions of miR-129-5p and apoptosis index Fas in IVDD tissues were determined using RT-PCR. NP cell apoptosis rate was detected *via* TUNEL assay. NP cells were extracted from IVDD tissues for primary culture. Subsequently, the cells were transfected with miR-129-5p inhibitor or mimic to inhibit or overexpress miR-129-5p, respectively. Furthermore, the changes in the JNK pathway indexes and apoptosis indexes were detected using Western blotting. In IVDD group, the expression of miR-129-5p was significantly down-regulated, while the transcriptional level of Fas was up-regulated compared with those in control group. Pearson correlation analysis revealed a negative correlation between the expressions of miR-129-5p and Fas mRNA (r=-0.75, P<0.05). IVDD group exhibited significantly higher levels of serum TNF-α, IL-6 and IL-1 than control group. Subsequent TUNEL assay indicated that the apoptosis rate was evidently higher in IVDD group (60.6%) than control group (2.5%). The results of Western blotting showed that the protein expressions of JNK1, JNK2 and Fas remarkably rose in IVDD group compared with those in control group. However, they declined remarkably in miR-129-5p mimic group compared with those in control group. Furthermore, such trends were significantly reversed in miR-129-5p inhibitor group. MiR-129-5p was significantly down-regulated in IVDD, whose overexpression has anti-inflammatory and anti-apoptotic effects.

Keywords: MiR-129-5p, Intervertebral disc degeneration (IVDD), Inflammation, Apoptosis.

1. Introduction

Intervertebral disc degeneration (IVDD) induced lumbago has become a serious global problem, affecting people's life quality [1]. Histologically, IVD consists of three interdependent and structurally different parts: (1) nucleus pulposus (NP) containing a large number of extracellular matrixes (ECM), such as proteoglycan and collagen II, (2) annulus fibrosus (AF) composed of a large amount of collagen I, and (3) upper and lower cartilage endplate (CEP) responsible for regulating the diffusion of nutrients in the vertebral body and IVD [2]. Due to these complex structures, IVD possesses unique biomechanical properties to keep the flexibility and mechanical stability of the spine. The changes in IVD will alter the arrangement and mechanical environment of the vertebral body, facet joints, spinal ligaments and muscles, eventually leading to spinal pain [3]. In degenerated IVD, a series of inflammatory factors and chemical mediators can be produced. Meanwhile, they can trigger pain in peripheral nerve endings and induce the growth of blood vessels and nerves into IVD circular defects [4].

Previous researches have found that IVDD is a chronic process of excessive destruction of ECM. It is also considered as the main cause of lumbago as well [5]. However, the potential mechanism of IVDD has not been fully clarified. Recent studies have shown that apoptosis is an important form of IVD cell death [6]. The reason is that apoptosis-induced cell loss contributes to the degradation of ECM and plays an important role during IVDD [7]. However, the specific mechanism of apoptosis in degenerated IVD remains unknown.

Microribonucleic acids (miRNAs) are a class of endogenous non-coding RNA molecules with about 22 nucleotides in length. They can regulate gene expression through base pairing with the 3'-untranslated region of target mRNAs [8,9]. MiRNAs have been confirmed involved

* Corresponding author.
E-mail address: dgaoxi1973@126.com (X. Gao).
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in several life processes, such as cell proliferation, apoptosis and invasion. Meanwhile, their dysregulation is correlated with many human diseases, including IVDD [10,11]. For example, miR-129-5p inhibits Fas-mediated apoptosis of various human cells *via* regulating Fas [12]. However, the specific role of miR-129-5p during the progression of IVDD has not been fully elucidated.

In this study, inflammatory factors and NP cell apoptosis were used as indexes of IVDD. The indexes of the c-Jun N-terminal kinase (JNK) signaling pathway were analyzed as well. All our findings might help to explore the potential mechanism of miR-129-5p in IVDD.

2. Materials and Methods

2.1. Animal Modeling

A total of 20 male Sprague-Dawley rats (4 weeks old, 200 g) were randomly selected and divided into two groups, including control group (n=10) and IVDD group (n=10). The IVDD model was successfully established *via* endplate injection. In brief, a 21 G puncture needle was locally punctured at the upper and lower endplates of the L5/6 IVD into the bone marrow cavity. From there, 30 μL of absolute ethanol was injected under pressure to block the nutrient penetration from the cartilage endplate to AF and NP, resulting in metabolic disorders. In control group, the same amount of normal saline was injected. After 1 month, subsequent experiments were performed. This study was approved by the Animal Ethics Committee of Heilongjiang University of Chinese Medicine Animal Center.

2.2. Cell Culture

NP cells were sterilely isolated from IVDD tissues. Briefly, IVDD tissues were washed with phosphate-buffered saline 3 times and cut into fragments the size of mung bean. After treatment with 0.25% trypsin for 30 min, the tissues were digested with collagenase II (Thermo Fisher Scientific, Waltham, MA, USA) at 37°C for 3-4 h. Subsequently, they were re-suspended in Dulbecco's Modified Eagle's Medium (DMEM/F12) (Gibco, Rockville, MD, USA) containing 15% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 μg/mL streptomycin, 100 U/mL penicillin and 1% L-glutamine, and incubated in an incubator with 5% CO_2 at 37°C. After culturing for 2 d, the cells were collected for subsequent experiments.

2.3. MiR-129-5p Interference and Cell Transfection

The miR-129-5p sequence (5'-CGGCAGGCATTAGAGATGAACAGCA-3') was knocked out in a targeted way. The hairpin siRNA without homology to human genes (RiboBio, Guangzhou, China) was used as a negative control. 1.5×10^5 cells were inoculated into a 35 mm culture dish for 24 h. Cell transfection was performed according to the instructions of Lipofectamine 2000 reagent (Thermo Fisher Scientific, Waltham, MA, USA). At 24 h, the transfection efficiency was verified, and total RNA was extracted from cells.

The miR-129-5p inhibitor and mimic at a final concentration of 2 μg/mL (RiboBio, Guangzhou, China) were transfected into NP cells using the transfection reagent. Transfected cells for 48 h were selected for the following experiments.

2.4. Detection of MiR-129-5p and Fas mRNA Levels Via Fluorescence Quantitative Real Time-Polymerase Chain Reaction (RT-PCR)

Total RNA was first isolated using TRIzol reagent (TaKaRa, Dalian, China). Subsequently, extracted RNA was synthesized into the first-strand complementary deoxyribose nucleic acid (cDNA) using the reverse transcription kit (Invitrogen, Carlsbad, CA, USA). RT-PCR was then performed using the standard SYBR Green PCR kit in the StepOne Plus System (Applied Biosystems, Foster City, CA, USA). Primers used in this study were as follows: MiR-129-5p: sense strand: 5'-ACCCAGTGCGATTTGTCA-3', antisense strand: 5'-ACTGTACTGGAAGATGGACC-3'. U6: sense strand: 5'- CTCGCTTCGGCAGCACA -3', antisense strand: 5'- AACGCTTCACGAATTTGCGT -3'; Fas: sense strand: 5'-CTGCATCATGATGGCCAATTCTGC3-3', antisense strand: 5'-ATGACACTAAGTCAAGTTAAAGGC-3'. GAPDH: sense strand: 5'-GGGAGCCAAAAGGGTCAT-3', antisense strand: 5'-GAGTCCTTCCACGATACCAA-3'. The expression of miR-26 was detected using TaqMan microRNA reverse transcription kit and TaqMan Master Mix II (Applied Biosystems, Foster City, CA, USA). The fold change of gene expression was calculated by the $2^{-\Delta\Delta Ct}$ method.

2.5. Determination of Protein Expression Using Western Blotting

40 μg of protein samples were separated and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After sealing with 5% skimmed milk at room temperature for 1 h, the membranes were incubated with primary antibodies of JNK1 (1:1,000, Proteintech, Rosemont, IL, USA), JNK2 and Fas (1:1,000, Abcam, Cambridge, MA, USA) overnight. After washing with Tris-Buffered Saline and Tween (TBST), the membranes were incubated again with corresponding horseradish peroxidase-labeled secondary antibodies (1:5,000, Shanghai Beyotime Biotechnology Co., Ltd., Shanghai, China). Immuno-reactive bands were visualized with the enhanced chemiluminescence (ECL) system (Bio-Rad, Hercules, CA, USA), and gray value was analyzed using a gel analyzer. The ratio of the gray value of target protein to that of corresponding internal reference indicated the relative expression of target protein.

2.6. Statistical Analysis

GraphPad Prism 5.01 software (La Jolla, CA, USA) was used for all statistical analysis. Measurement data were expressed as $\bar{\chi}\pm s$. The differences in indexes between the two groups were compared using the independent-samples *t*-test. The correlation between expressions of miR-129-5p and Fas in IVDD rats was analyzed *via* Pearson correlation analysis. $P<0.05$ was considered statistically significant.

3. Results

3.1. MiR-129-5p and Fas mRNA Levels in IVDD Tissues Detected Via RT-PCR

To investigate the biological role of miR-129-5p in IVDD, the expressions of miR-129-5p and Fas were determined in normal and IVDD rats *via* qRT-PCR. It was found that the expression of miR-129-5p was significantly down-regulated, while the transcriptional level of Fas was

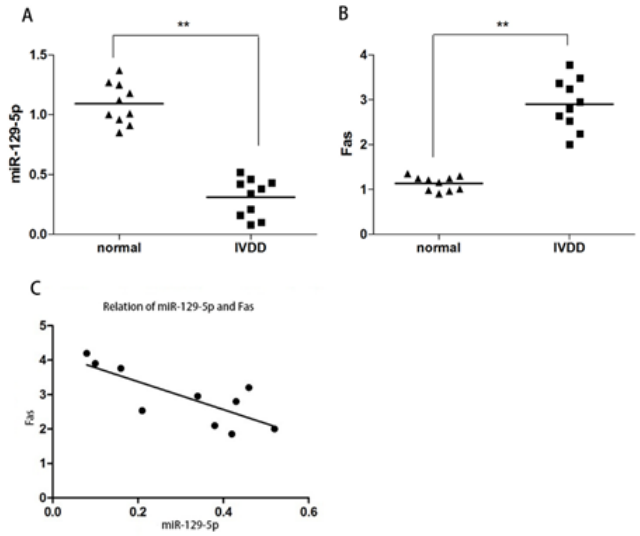

Fig. 1. Differences in miR-129-5p and Fas mRNA levels between control group and IVDD group. (A) and (B) Levels of miR-129-5p and Fas mRNA in IVD detected *via* RT-PCR. (C) Correlation between miR-129-5p and Fas mRNA expressions analyzed using Pearson correlation analysis. $^{**}P<0.01$ *vs.* control group.

up-regulated in IVDD tissues ($P<0.05$; Figure 1). According to the Pearson correlation analysis, there was a negative correlation between expressions of miR-129-5p and Fas mRNA ($r=-0.75$, $P<0.05$). It can be seen that miR-129-5p and Fas are involved in the progression of IVDD.

3.2. Levels of Serum Inflammatory Factors in Both Groups

IVDD group exhibited significantly higher levels of serum tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1 than control group ($P<0.05$) (Table 1).

3.3. Apoptosis Rate of NP Cells in Both Groups Determined Using TUNEL Assay

The results of RT-PCR demonstrated that miR-129-5p and Fas were involved in the progression of IVDD. To verify such a conclusion at the functional level, the apoptosis of NP cells was detected using TUNEL assay in both groups. The results indicated that the apoptosis rate was higher in IVDD group (60.6%) than in control group (2.5%) ($P<0.05$) (Figure 2).

3.4. Levels of JNK Pathway and Apoptosis Protein Detected Via Western Blotting

Total protein was first extracted from IVD tissues, and protein expressions of JNK1, JNK2 and Fas were determined using Western blotting. As shown in Figure 3, the protein expressions of JNK1, JNK2 and Fas remarkably rose in IVDD group compared with those in control group ($P<0.05$). It can be seen that the JNK pathway and apoptotic pathway are significantly activated in the IVDD model. Meanwhile, they may play important roles in the occurrence and development of IVDD.

3.5. Effects of MiR-129-5p on JNK Pathway and Apoptosis Protein in NP Cells Detected In Vitro

To verify whether the anti-inflammatory and anti-apoptotic effects of miR-129-5p on IVDD are realized *via* the JNK signaling pathway, NP cells were extracted from IVDD tissues. Subsequently, the cells were transfected with the inhibitor or mimic to interfere with the expression of miR-129-5p. The changes in JNK pathway and apoptosis protein were then explored. As shown in Figure 4, the protein expressions of JNK1, JNK2 and Fas rose remarkably in miR-129-5p inhibitor group, and such trends were significantly reversed in miR-129-5p mimic group ($P<0.05$).

4. Discussion

Currently, studies have demonstrated that the occurrence and development of IVDD are closely related to the

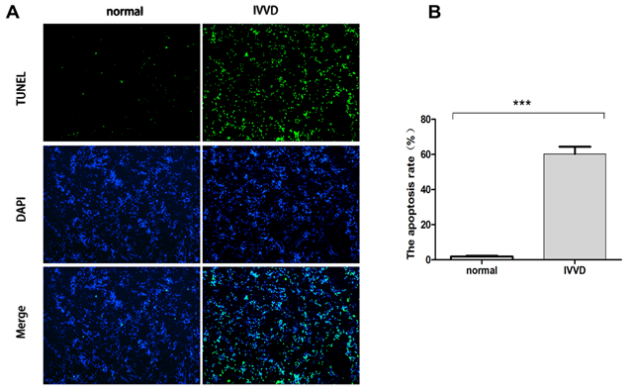

Fig. 2. Apoptosis rate of NP cells in both groups was determined using TUNEL assay. (A) Green fluorescence: apoptotic nuclei, blue fluorescence: DAPI-stained nuclei. (magnification: 400×). (B) Apoptosis rate of NP cells in both groups. $^{***}P<0.001$.

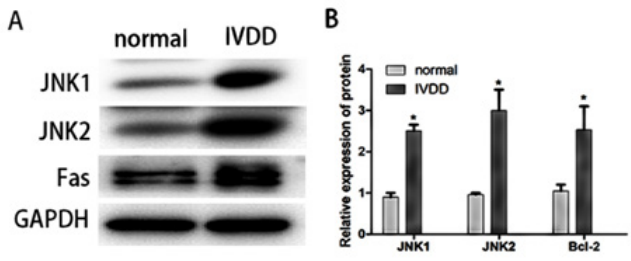

Fig. 3. Levels of JNK pathway and apoptosis protein detected *via* Western blotting. (A) Levels of JNK pathway and apoptosis protein detected *via* Western blotting. (B) Differences in protein expressions between the two groups. Note: The protein expressions of JNK1, JNK2 and Fas remarkably rose in IVDD group compared with those in control group ($P<0.05$). $^{*}P<0.05$ *vs.* control group.

Table 1. Levels of serum inflammatory factors in both groups ($\bar{\chi}$±s).

Group	n	TNF-α (pg/mL)	IL-6 (pg/mL)	IL-1 (pg/mL)
Control group	10	12.34±1.88	10.58±0.51	8.22±0.35
IVDD group	10	29.06±3.65*	23.62±2.01*	19.54±1.62*

Note: $^{*}P<0.05$ *vs.* control group.

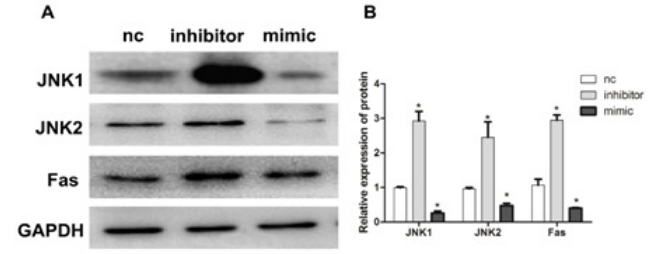

Fig. 4. Effects of miR-129-5p on JNK pathway and apoptosis protein in NP cells detected *in vitro*. (A) Levels of JNK pathway and apoptosis protein detected *via* Western blotting. (B) Differences in protein expressions between the two groups. Note: The protein expressions of JNK1, JNK2 and Fas remarkably rose in miR-129-5p inhibitor group, while they significantly declined in miR-129-5p mimic group ($P<0.05$). $^{*}P<0.05$ *vs.* control group.

production of various inflammatory cytokines and metabolites. During the process of IVDD, a large number of inflammatory, degradation and decomposition molecules will be produced, including proteolytic and degrading enzymes, oxygen free radicals, nitric oxide, ILs and prostaglandins [13]. Risbud and Shapiro [14] have found that spinal instability and structural changes caused by the increase in inflammatory cytokines and decrease in hydrophilic matrix molecules are the major causes of protrusion, sciatica and stenosis. These inflammatory factors mainly include IL-1, IL-6, TNF-α, lipopolysaccharides and reactive oxygen species. IL-1 plays a major role in degenerative musculoskeletal diseases. It increases the matrix penetration rate in IVD, reduces the proteoglycan synthesis rate of IVD cells, and induces the expression of prostaglandin E2. As a pro-inflammatory cytokine, TNF-α plays a key role in discogenic pain and sciatica [15]. In this study, the expressions of serum inflammatory factors were explored in IVDD rats. The results found that the levels of serum TNF-α, IL-6 and IL-1 significantly rose in IVDD group.

As a common form of programmed cell death, apoptosis is initiated upon the stimulation of inflammation, damage, DNA injury and oxidative stress. It is widely involved in the pathophysiological process of a variety of degenerative diseases, including IVDD [16]. Wei et al. [17] have found that bone morphogenetic protein-7 protects human IVD cells against apoptosis *in vitro*. Fas is a membrane-bound receptor, which binds to cells with Fas ligand to initiate apoptosis [18]. It has been demonstrated that Fas and its receptors are not carried in normal IVD cells. However, they begin to be expressed shortly after IVDD [19]. In the present study, the levels of miR-129-5p and Fas mRNA were detected *via* RT-PCR. The results revealed that miR-129-5p was remarkably down-regulated, while Fas mRNA was significantly up-regulated in IVDD rats. Meanwhile, there was a negative correlation between the two molecules. Histologically, the results of TUNEL assay manifested that the apoptosis of NP cells in IVDD group was evidently enhanced. The above findings suggest that miR-129-5p plays an important role in the apoptosis of NP cells in IVDD. Previous studies have shown that IVDD occurs due to excessive degradation and impaired synthesis of ECM secreted by NP cells [20-23]. Therefore, primary NP cells were extracted from IVDD tissues, and transfected with the inhibitor or mimic to interfere with the expression of miR-129-5p. Meanwhile, the changes in JNK pathway and apoptosis protein were explored. The results demonstrated that miR-129-5p regulated the apoptosis of NP cells through the JNK pathway.

Furthermore, Benakis et al. [24] have shown that the mitogen-activated protein kinase (MAPK) pathway is an important downstream inflammatory pathway in cartilage tissues. In the pathway, p38, extracellular signal-regulated kinase (ERK) and JNK are main members. According to the research by Li et al. [25] on cartilage degeneration, the MAPK pathway plays a key role in the inflammatory process and apoptosis. Ren et al. [26] have also found that miR-138 protects against H_2O_2-induced BV-2 cell apoptosis *via* regulating the MLK3/JNK/MAPK pathway. In this study, it was confirmed that miR-129-5p exerted an anti-apoptotic effect through the JNK pathway.

5. Conclusions

Altogether, these data demonstrate that miR-129-5p exhibits good anti-inflammatory and anti-apoptotic effects in the IVDD model. In addition, such a finding may provide a basis for the treatment of IVDD with miR-129-5p.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of Heilongjiang University of Chinese Medicine Animal Center.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Chen Li and Xi Gao designed the study and performed the experiments, Zhiwen Sun and Hongjun Lou collected the data, Yenong Sun and Chengyuan Li analyzed the data, Chen Li and Xi Gao prepared the manuscript. All authors read and approved the final manuscript.

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References

1. Chan SC, Ferguson SJ, Gantenbein-Ritter B (2011) The effects of dynamic loading on the intervertebral disc. Eur Spine J 20:1796-1812. doi: 10.1007/s00586-011-1827-1
2. Hanaei S, Abdollahzade S, Sadr M, Mirbolouk MH, Fattahi E, Khoshnevisan A et al (2020) The role of interleukin 4 and IL-4RA in intervertebral disc degeneration: investigation of single nucleotide polymorphisms in genes and a systematic review & meta-analysis of IL-4 expression level. Brit J Neurosurg 34:66-71. doi: 10.1080/02688697.2019.1698010

3. Sakai D, Andersson GB (2015) Stem cell therapy for intervertebral disc regeneration: obstacles and solutions. Nat Rev Rheumatol 11:243-256. doi: 10.1038/nrrheum.2015.13
4. O'Halloran DM, Pandit AS (2007) Tissue-engineering approach to regenerating the intervertebral disc. Tissue Eng 13:1927-1954. doi: 10.1089/ten.2005.0608
5. Le Maitre CL, Freemont AJ, Hoyland JA (2007) Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. Arthritis Res Ther 9:R45. doi: 10.1186/ar2198
6. Zhao CQ, Jiang LS, Dai LY (2006) Programmed cell death in intervertebral disc degeneration. Apoptosis 11:2079-2088. doi: 10.1007/s10495-006-0290-7
7. Zhang F, Zhao X, Shen H, Zhang C (2016) Molecular mechanisms of cell death in intervertebral disc degeneration (Review). Int J Mol Med 37:1439-1448. doi: 10.3892/ijmm.2016.2573
8. Gu SX, Li X, Hamilton JL, Chee A, Kc R, Chen D et al (2015) MicroRNA-146a reduces IL-1 dependent inflammatory responses in the intervertebral disc. Gene 555:80-87. doi: 10.1016/j.gene.2014.10.024
9. Wang C, Wang WJ, Yan YG, Xiang YX, Zhang J, Tang ZH et al (2015) MicroRNAs: New players in intervertebral disc degeneration. Clin Chim Acta 450:333-341. doi: 10.1016/j.cca.2015.09.011
10. Yun Z, Wang Y, Feng W, Zang J, Zhang D, Gao Y (2020) Overexpression of microRNA-185 alleviates intervertebral disc degeneration through inactivation of the Wnt/beta-catenin signaling pathway and downregulation of Galectin-3. Mol Pain 16:2068260961. doi: 10.1177/1744806920902559
11. Liu W, Xia P, Feng J, Kang L, Huang M, Wang K et al (2017) MicroRNA-132 upregulation promotes matrix degradation in intervertebral disc degeneration. Exp Cell Res 359:39-49. doi: 10.1016/j.yexcr.2017.08.011
12. He J, Xue R, Li S, Lv J, Zhang Y, Fan L et al (2015) Identification of the potential molecular targets for human intervertebral disc degeneration based on bioinformatic methods. Int J Mol Med 36:1593-1600. doi: 10.3892/ijmm.2015.2389
13. Molinos M, Almeida CR, Caldeira J, Cunha C, Goncalves RM, Barbosa MA (2015) Inflammation in intervertebral disc degeneration and regeneration. J R Soc Interface 12:20141191. doi: 10.1098/rsif.2014.1191
14. Risbud MV, Shapiro IM (2014) Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol 10:44-56. doi: 10.1038/nrrheum.2013.160
15. Sinclair SM, Shamji MF, Chen J, Jing L, Richardson WJ, Brown CR et al (2011) Attenuation of inflammatory events in human intervertebral disc cells with a tumor necrosis factor antagonist. Spine 36:1190-1196. doi: 10.1097/BRS.0b013e3181ebdb43
16. Rannou F, Lee TS, Zhou RH, Chin J, Lotz JC, Mayoux-Benhamou MA et al (2004) Intervertebral disc degeneration: the role of the mitochondrial pathway in annulus fibrosus cell apoptosis induced by overload. Am J Pathol 164:915-924. doi: 10.1016/S0002-9440(10)63179-3
17. Wei A, Brisby H, Chung SA, Diwan AD (2008) Bone morphogenetic protein-7 protects human intervertebral disc cells in vitro from apoptosis. Spine J 8:466-474. doi: 10.1016/j.spinee.2007.04.021
18. Wang F, Jiang JM, Deng CH, Wang FL, Fu ZZ, Zhang ZF (2011) Expression of Fas receptor and apoptosis in vertebral endplates with degenerative disc diseases categorized as Modic type I or II. Injury 42:790-795. doi: 10.1016/j.injury.2011.01.034
19. Cui LY, Liu SL, Ding Y, Huang DS, Ma RF, Huang WG et al (2007) IL-1beta sensitizes rat intervertebral disc cells to Fas ligand mediated apoptosis in vitro. Acta Pharmacol Sin 28:1671-1676. doi: 10.1111/j.1745-7254.2007.00642.x
20. Sakai D, Nakamura Y, Nakai T, Mishima T, Kato S, Grad S et al (2012) Exhaustion of nucleus pulposus progenitor cells with ageing and degeneration of the intervertebral disc. Nat Commun 3:1264. doi: 10.1038/ncomms2226
21. Azizi Dargahlou S, Iriti M, Pouresmaeil M, Goh LPW. (2023) MicroRNAs; their therapeutic and biomarker properties. Cell Mol Biomed Rep 3(2): 73-88. doi: 10.55705/cmbr.2022.365396.1085
22. Kanwal N, Al Samarrai O, Al-Zaidi HMH, Mirzaei A, Heidari M. (2023). Comprehensive analysis of microRNA (miRNA) in cancer cells. Cell Mol Biomed Rep 3(2): 89-97. doi: 10.55705/cmbr.2022.364591.1070.
23. Sasani S, Rashidi Monfared S, Mirzaei AR (2024). Identification of some Echinophora platyloba miRNAs using computational methods and the effect of these miRNAs in the expression of TLN2 and ZNF521 genes in different human body organs. Cell Mol Biomed Rep 4(1): 43-53. doi: 10.55705/cmbr.2023.386145.1100.
24. Benakis C, Bonny C, Hirt L (2010) JNK inhibition and inflammation after cerebral ischemia. Brain Behav Immun 24:800-811. doi: 10.1016/j.bbi.2009.11.001
25. Li JK, Nie L, Zhao YP, Zhang YQ, Wang X, Wang SS et al (2016) IL-17 mediates inflammatory reactions via p38/c-Fos and JNK/c-Jun activation in an AP-1-dependent manner in human nucleus pulposus cells. J Transl Med 14:77. doi: 10.1186/s12967-016-0833-9
26. Ren R, Chen SD, Fan J, Zhang G, Li JB (2018) miRNA-138 regulates MLK3/JNK/MAPK pathway to protect BV-2 cells from H2O2-induced apoptosis. Bratisl Med J 119:284-288. doi: 10.4149/BLL_2018_053