

MicroRNA-25 protects nucleus pulposus cells against apoptosis via targeting SUMO2 in intervertebral disc degeneration

CHANGBIN LEI¹⁻³, JIAN LI², GUANG TANG² and JIONG WANG²

Departments of ¹Clinical Medical Research Center and ²Heavy Metal Pollution and Cancer Prevention Technology Research Center, Affiliated Hospital of Xiangnan University (Clinical College), Chenzhou, Hunan 423000;

³Department of Orthopedics, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong 510630, P.R. China

Received July 9, 2020; Accepted June 24, 2021

DOI: 10.3892/mmr.2021.12363

Abstract. It has been reported that microRNA (miRNA/miR)-25 is downregulated in patients with intervertebral disc degeneration (IVDD). However, the potential role of miR-25 in IVDD remains unclear. Therefore, the present study aimed to investigate the effects of miR-25 on human intervertebral disc nucleus pulposus cells (NPCs). The expression levels of miR-25 and those of small ubiquitin-related modifier 2 (SUMO2) were determined in human nucleus pulposus (NP) tissues by reverse transcription-quantitative PCR (RT-qPCR) and western blot analyses. Subsequently, the potential interaction between miR-25 and SUMO2 was validated via dual-luciferase reporter assay and RNA pull-down assay with biotinylated miRNA. The effects of miR-25 on NPC proliferation and apoptosis were evaluated using Cell Counting Kit-8 assay, 5-ethynyl-2'-deoxyuridine incorporation assay, and flow cytometry. The results showed that miR-25 was downregulated in patients with IVDD. In addition, miR-25 increased the proliferation of NPCs and inhibited their apoptosis. Furthermore, the current study verified that miR-25 could directly target SUMO2 and regulate its expression via the p53 signaling pathway. Additionally, the effects of miR-25 on NPCs were abrogated following SUMO2 overexpression. Overall, the results of the present study demonstrated that miR-25 could promote the proliferation and inhibit the apoptosis of NPCs via targeting SUMO2, suggesting that miR-25 may be a potential target in the treatment of IVDD.

Introduction

Intervertebral disc degeneration (IVDD) is the leading cause of vertebral disc herniation, spondylolisthesis, spinal canal stenosis, and other spinal degenerative diseases (1). Due to its high morbidity and disability rate, IVDD imposes a heavy socioeconomic burden (2). Although patients with IVDD respond well to the main treatment approaches, including nucleus pulposus (NP) allograft, spinal canal decompression and spinal fusion, the poor long-term treatment efficacy represents a major cause of failure (3).

To the best of our knowledge, the apoptosis of NP cells (NPCs), microfractures caused by excessive pressure, extracellular matrix degradation and the abnormal expression of inflammatory factors, can disrupt the dynamic balance of anabolism and catabolism of the intervertebral disc matrix, thus resulting in the occurrence and development of IVDD (4,5). However, the cellular and molecular mechanisms underlying IVDD remain unclear. Therefore, more experiments are needed to investigate the regulatory mechanism of NPCs in the pathophysiology of IVDD.

Non-coding RNAs, which can regulate gene expression, are involved in several pathophysiological processes of intervertebral disc cells (6,7). MicroRNAs (miRNAs/miRs) are short, single-stranded non-coding RNAs that bind to the 3'-untranslated region (3'-UTR) of their target mRNAs to inhibit their translation or promote degradation, thus regulating cell differentiation, proliferation and survival (8). It has been reported that several miRNAs are differentially expressed in intervertebral disc tissues with different degrees of degeneration, and are involved in the regulation of multiple physiological processes, such as NPC apoptosis and proliferation, and the degradation of extracellular matrix (9). Further investigations on the key miRNA molecules regulating NPCs in IVDD would provide novel approaches for the diagnosis and treatment of IVDD.

miRNAs are non-coding RNAs, 18-22 nucleotides in length, that regulate the expression of their target genes by specific binding to their 3'-UTR (10). Emerging evidence has suggested that miRNAs are involved in several cellular processes, including cell proliferation, apoptosis, differentiation and invasion (11-13).

A recent study demonstrated that miR-25 could promote the proliferation of breast cancer cells via targeting B-cell

Correspondence to: Dr Changbin Lei, Department of Clinical Medical Research Center, Affiliated Hospital of Xiangnan University (Clinical College), 25 Renmin West Road, Beihu, Chenzhou, Hunan 423000, P.R. China
E-mail: leichangbin@126.com

Abbreviations: IVDD, intervertebral disc degeneration; SUMO2, small ubiquitin-related modifier 2; NPCs, nucleus pulposus cells; NP, nucleus pulposus; SD, standard deviation; RT-qPCR, reverse transcription-quantitative PCR; EdU, 5-ethynyl-2'-deoxyuridine

Key words: intervertebral disc degeneration, microRNA-25, nucleus pulposus cells, small ubiquitin-related modifier 2

translocation gene 2 (14). In addition, miR-25 promoted the malignant phenotype of retinoblastoma cells via regulating the phosphatase and tensin homolog (PTEN)/Akt pathway (15). Another study showed that miR-25 could facilitate the invasion of human non-small cell lung cancer cells through cadherin 1 (16). Of note, a previous microarray analysis revealed that miR-25 was downregulated in patients with IVDD (17). However, the mechanism underlying the effect of miR-25 on IVDD remains to be elucidated.

The small ubiquitin-related modifier (SUMO)2 protein is a member of the SUMO family, also including SUMO1, SUMO2 and SUMO3, which is involved in post-translational modification by conjugating with its target proteins (18). SUMO2 serves an important role in the regulation of several target molecules (19,20). The aim of the present study was to investigate whether the miR-25-mediated blocking of the p53 signaling pathway via SUMO2 could attenuate the apoptosis of intervertebral disc-derived primary human NPCs.

Materials and methods

Tissue collection. NP tissues were collected from 30 patients with IVDD (age, 36-65 years; 18 males and 12 females) from March 2019 to January 2020, who underwent lumbar disc herniation surgery at the First Affiliated Hospital of Jinan University (Guangzhou, China). The degree of IVDD in each operation section was determined according to the Pfirrmann classification score, which was divided to grades I-V based on Magnetic Resonance Imaging (21). Control samples were obtained from 30 age- and sex-matched patients with fresh traumatic lumbar fracture, who underwent anterior decompressive surgery due to neurological deficits. The current study was approved by the Ethics Committee of the Affiliated Hospital of Xiangnan University (approval no. XNEC-2019036; Chenzhou, China). All subjects provided written informed consent prior to enrollment.

Cell isolation and culture. The NP tissues of Pfirrmann classification grade II were rinsed twice with PBS and cut into 1-mm³ pieces followed by digestion with trypsin. The NP tissues were carefully examined using a dissecting microscope to remove any adherent tissues, such as the annulus fibrosus, cartilage endplate and ligaments. Subsequently, the samples were digested following incubation with 0.25% type I collagenase at 37°C overnight. The isolated NPCs were maintained in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 100 mg/ml streptomycin, 100 U/ml penicillin, and 1% L-glutamine (Gibco; Thermo Fisher Scientific, Inc.) at 37°C.

Cell transfection. The miR-25 mimic (miR-25 mimic), negative control mimic (mimic NC), miR-25 inhibitor (miR-25 inhibitor), negative control inhibitor (inhibitor NC), as well as the blank control (blank), were obtained from Shanghai GenePharma Co., Ltd. For SUMO2 overexpression (SUMO2), SUMO2 gene (NM_006937) or negative control, which was a scramble sequence (vector), were sub-cloned into the GV365 vector (Shanghai GeneChem Co., Ltd.). miR-25 mimic (50 nM), mimic NC (50 nM), miR-25 inhibitor (50 nM), inhibitor NC (50 nM) and SUMO2 (50 nM) were co-transfected into 293

cells (American Type Culture Collection) with expression vectors. Cell transfection (1x10³ cells) was performed as previously described (22) using Lipofectamine[®] 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 48 h, according to the manufacturer's instructions, when cells reached 30-50% confluence. The subsequent experimentation were performed 48 h post-transfection. For transient transfection, the following sequences were used: miR-25 mimic, 5'-AGGCGGAGACUUGGGCAAUUG-3'; miR-25 inhibitor, 5'-AGGCGGAGACUUGGGCAAUUG-3'; and NC, 5'-UUGUACUACACAAAAGUACUG-3'.

Dual-luciferase reporter assay. Targetscan (targetscan.org/vert_72/) was used for prediction of miR-25 target genes. For the dual-luciferase reporter assay, luciferase plasmids containing the sequences of SUMO2 3'-UTR with a wild-type (WT) or mutant (MUT) binding site for miR-25 were synthesized by Wuhan GeneCreate Biological Engineering Co., Ltd. NPCs were co-transfected with the luciferase reporter vectors (Promega Corporation) encompassing the wild-type (WT) or mutant (Mut) SUMO2 3'-UTR and miR-25 mimic using Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following 48 h culture at room temperature, cell lysates were collected and analyzed for firefly and *Renilla* luciferase activity using a dual-luciferase assay kit (Beijing Solarbio Science & Technology Co., Ltd.) in dual-luciferase reporter assay system (Promega Corporation). All experiments were independently repeated in triplicate.

RNA pull-down assay. Cell lysates extracted using RIPA lysis buffer were employed for RNA pull-down assay and using a Pierce[™] Magnetic RNA-Protein Pull-Down kit (cat. no. #20164; Thermo Fisher Scientific, Inc.). Biotin-labeled RNAs (bio-miR-25) were reverse-transcribed, lysed in RNase-free cell lysis solution at 4°C and treated with RNase-free DNase I. Cell lysates were incubated with M-280 streptavidin magnetic beads (Invitrogen; Thermo Fisher Scientific, Inc.) overnight at 4°C according to the manufacturer's protocol. Next, the beads were washed with high salt buffer. Following centrifugation (1,500 x g; 10 min; 4°C), the pellet was lysed with TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The enrichment of SUMO2 mRNA in co-precipitated RNAs was determined by RT-qPCR.

RT-qPCR analysis. Total RNA was extracted from transfected NPCs using TRIzol[®] reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. For RT, the isolated RNA was reverse-transcribed into cDNA using a Reverse Transcription kit (Takara Bio, Inc.) according to the manufacturer's protocol. To quantify the expression levels of SUMO2 and p53, qPCR analysis was carried out with the TB Green[®] Premix Ex Taq[™] kit (cat. no. RR420A; Takara Bio, Inc.). GAPDH served as the internal control. In addition, miR-25 was reverse-transcribed into cDNA using the Revert Aid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The expression of miR-25 was quantified using qPCR with the Mir-X[™] miRNA qRT-PCR TB Green[®] Kit (cat. no. 638314; Clontech Laboratories, Inc.). U6 served as the internal control

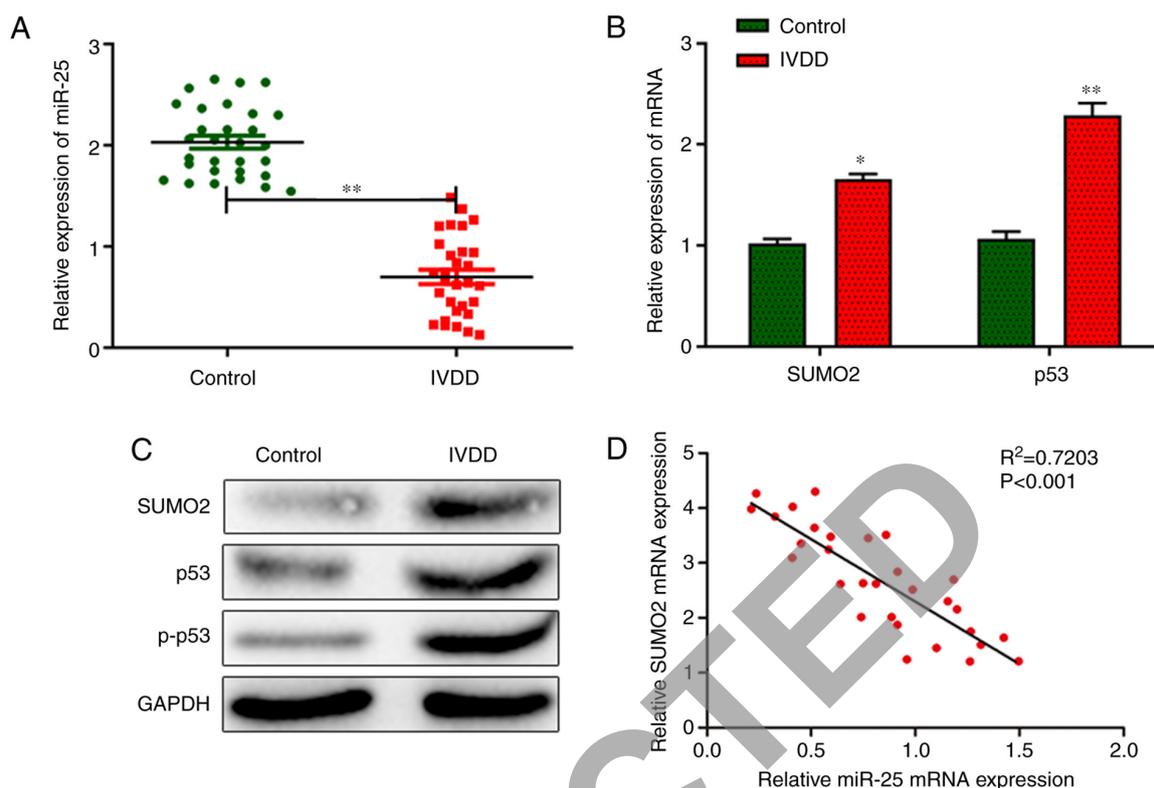


Figure 1. miR-25 is downregulated in patients with IVDD. (A) miR-25 expression levels in NP tissues of patients with IVDD and controls (n=30) were determined by RT-qPCR analysis. **P<0.01. (B and C) mRNA and protein levels of SUMO2, p53 and p-p53 were assessed using RT-qPCR and western blot analyses, respectively. (D) Results of the correlation analysis between miR-25 and SUMO2 expression levels in NP tissues obtained from 30 patients with IVDD. Data are expressed as the mean \pm SD (n=3). *P<0.05 and **P<0.01 vs. control. IVDD, intervertebral disc degeneration; NP, nucleus pulposus; RT-qPCR, reverse transcription-quantitative PCR; SUMO2, small ubiquitin-related modifier 2; p-, phosphorylated; miR, microRNA.

for miRNA expression. The thermocycling conditions used were as follows: 95°C for 10 min; 40 cycles of 95°C for 15 sec followed by 60°C for 30 sec. The gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method (23). The experiments were performed in triplicate. The primer sequences used were as follows: miR-25 forward, 5'-AGGCGGAGACTTGGGCAATTG-3'; SUMO2 forward, 5'-GGCAACCAATCAACGAAACAG-3' and reverse, 5'-TGCTGGAACACATCAATGTATC-3'; p53 forward, 5'-GACGCTGCCCCACCATGAG-3' and reverse, 5'-ACCACCACGCTGTGCCGAAA-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; and GAPDH forward, 5'-CCACGAACTACCTTCAACTC-3' and reverse, 5'-TCA TACTCCTGCTGCTTGCTGATCC-3'.

Western blot analysis. Total proteins (20 μ g) were extracted from transfected NPCs using RIPA lysis buffer and the protein concentration was then quantified using the BCA Protein Assay kit (both Beyotime Institute of Biotechnology). The protein samples were separated via SDS-PAGE on 12% gel and were then transferred onto PVDF membranes and blocked with 5% non-fat milk for 2 h at room temperature followed by incubation with primary antibodies at 4°C overnight and horseradish peroxidase-conjugated secondary antibodies (cat. no. ab150077; 1:1,000; Abcam) at room temperature for 2 h. The protein blots were visualized utilizing enhanced chemiluminescence (Thermo Fisher Scientific, Inc.) and quantified using ImageJ 1.8.0 software (National Institutes of

Health). The specific primary antibodies used were as follows: SUMO2 (cat. no. ab234859; 1:1,000), phosphorylated (p)-p53 (cat. no. ab33889; 1:1,000), p53 (cat. no. ab32389; 1:1,000), Bax (cat. no. ab263897; 1:1,000), Bcl-2 (cat. no. ab32124; 1:1,000) and GAPDH (cat. no. ab9485; 1:1,000; all Abcam).

Cell proliferation assay. For the Cell Counting Kit-8 (CCK-8) assay, CCK-8 reagent (10 μ l; Beyotime Institute of Biotechnology) was added into each well at 24, 48 and 72 h after cell transfection. Following incubation for 4 h, the number of surviving cells was evaluated by measuring the absorbance of each well at a wavelength of 450 nm. For the 5-ethynyl-2'-deoxyuridine (EdU) incorporation assay, transfected cells were exposed to EdU solution (500 μ l; Guangzhou RiboBio Co., Ltd.). Following incubation for 2 h, cells were fixed with 4% formaldehyde solution for 30 min at room temperature, permeabilized with 0.5% Triton X-100 for 10 min and observed under a fluorescence microscope (Thermo Fisher Scientific, Inc.). The experiments were performed in triplicate.

Cell apoptosis assay. Cell apoptosis (early + late) was assessed using flow cytometry. Briefly, transfected NPCs were seeded into 24-well plates (1×10^3 cells) and incubated for 24 h. Subsequently, cells were washed twice with PBS. Following digestion, centrifugation at 1,000 \times g for 5 min at room temperature and washing with PBS, cells were stained with the Annexin V-FITC Apoptosis Detection kit (BD Biosciences) in

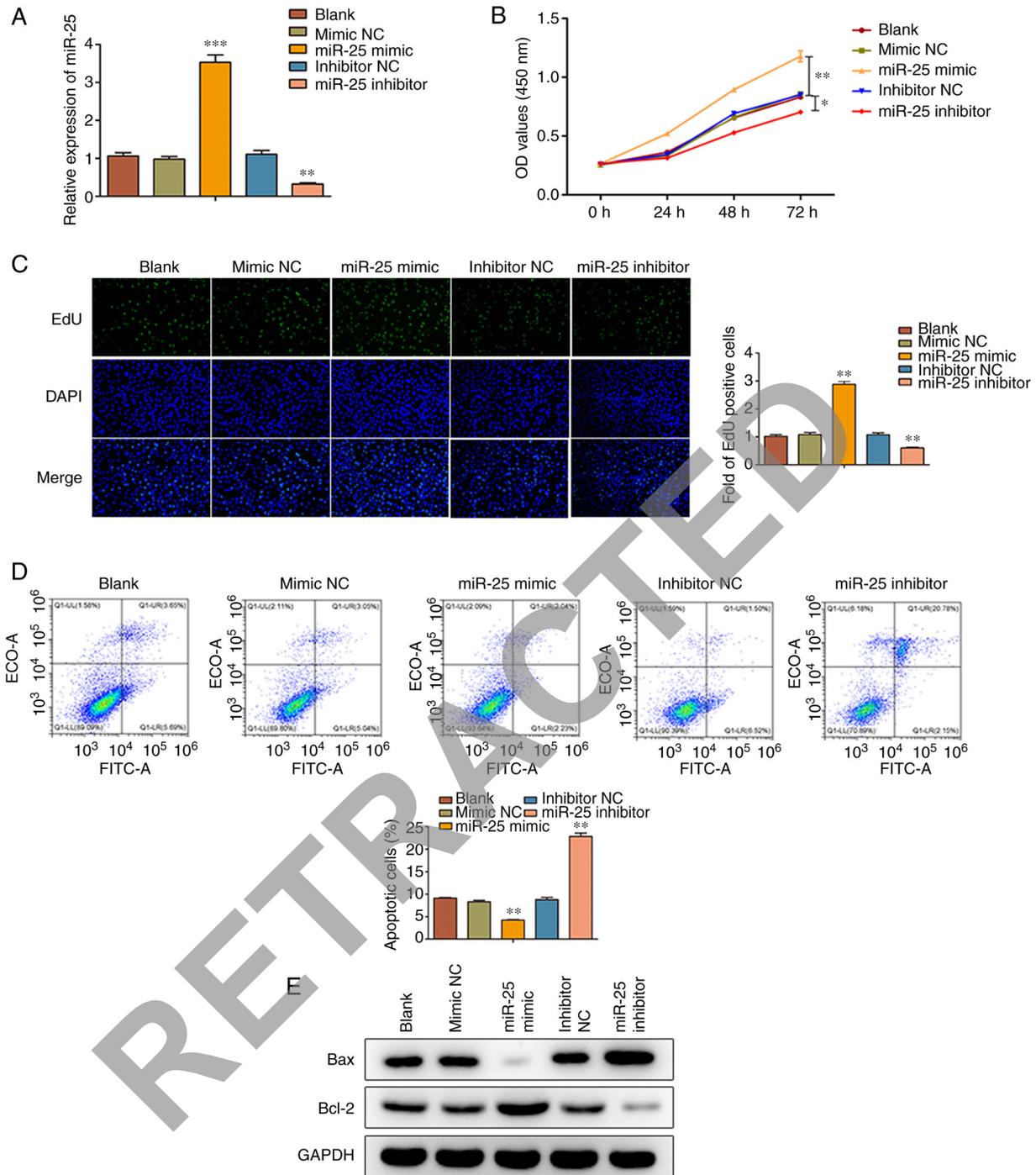


Figure 2. miR-25 promotes the proliferation and inhibits the apoptosis of human NPCs. (A) miR-25 expression was determined using reverse transcription-quantitative PCR assay. (B and C) Cell Counting Kit-8 and EdU assays were performed to evaluate cell proliferation. Magnification, $\times 200$. (D) Apoptosis rate was calculated using flow cytometry. (E) Western blot analysis of the protein expression levels of Bax and Bcl-2 in human NPCs following miR-25 overexpression or silencing. Data are expressed as the mean \pm SD ($n=3$). * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ vs. blank. NPCs, nucleus pulposus cells; miR, microRNA; NC, negative control; EdU, 5-ethynyl-2'-deoxyuridine.

the dark for 15 min. Finally, the apoptosis rate was analyzed utilizing the FACSCalibur flow cytometer (BD Biosciences) and BD Accuri C6 1.0.264.21 software (BD Biosciences). The experiment was repeated three times.

Statistical analysis. All experiments were performed in triplicate. All data are expressed as the mean \pm SD. Correlation analysis was carried out using the Spearman's rank test. The significant differences between two groups were evaluated using an unpaired

Student's t-test, while those among multiple groups were analyzed with ANOVA followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-25 is downregulated in patients with IVDD. The expression levels of miR-25 were significantly decreased in the NP tissues from patients with IVDD compared with controls

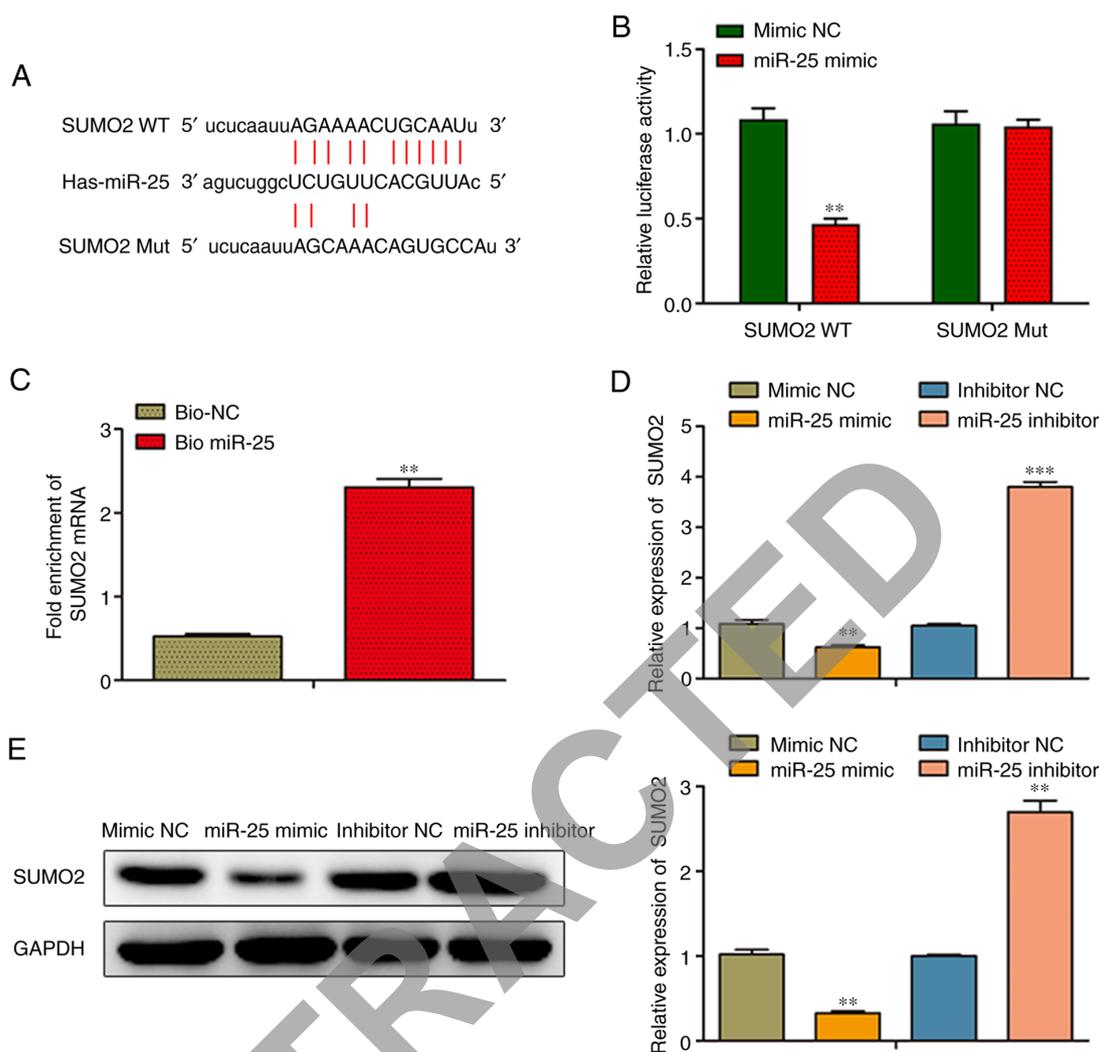


Figure 3. SUMO2 is directly targeted by miR-25 in human NPCs. (A) Sequence alignment between miR-25 with SUMO2. (B) Luciferase reporter assay results in NPCs co-transfected with miR-25 mimic or mimic NC and WT or Mut luciferase vector SUMO2. (C) Enrichment of SUMO2 mRNA was detected by bio-miR-25 pull-down assay in NPCs. (D and E) mRNA and protein expression levels of SUMO2 in NPCs transfected with miR-25 mimic, inhibitor or NCs. Data are expressed as the mean \pm SD (n=3). **P<0.01 and ***P<0.001. SUMO2, small ubiquitin-related modifier 2; NPCs, nucleus pulposus cells; NC, negative control; miR, microRNA; WT, wild-type; Mut, mutant; bio-, biotinylated; UTR, untranslated region.

(Fig. 1A). In addition, the mRNA and protein expression levels of SUMO2, p53 and p-p53 were markedly higher in the IVDD group compared with the control group (Fig. 1B and C). Additionally, bivariate correlation analysis showed that the mRNA expression of miR-25 was negatively correlated with that of SUMO2 in NP tissues of patients with IVDD (Fig. 1D).

miR-25 promotes the proliferation and inhibits the apoptosis of human NPCs. To further investigate the effects of miR-25, human NPCs were transfected with miR-25 mimic or inhibitor to overexpress or knock down miR-25 expression, respectively (Fig. 2A). Cell proliferation was then assessed using a CCK-8 assay, demonstrating that the proliferation rate of NPCs was markedly increased and attenuated following miR-25 overexpression and silencing, respectively, in a time-dependent manner (Fig. 2B). Additionally, EdU assay further confirmed the pro- and anti-proliferative effects of miR-25 overexpression and silencing, respectively (Fig. 2C). Following transfection, the NPC apoptosis rate was further evaluated by flow cytometry. Therefore, miR-25 overexpression suppressed

the apoptosis rate of NPCs, which was elevated after cell transfection with miR-25 inhibitor (Fig. 2D). Furthermore, the protein expression levels of Bax and Bcl-2 were determined by western blot analysis. The results showed that the protein levels of the pro-apoptotic protein Bax were markedly decreased, while those of the anti-apoptotic protein Bcl-2 were elevated in the miR-25 mimic group. However, miR-25 silencing exerted the opposite effects (Fig. 2E).

SUMO2 is a direct target of miR-25 in human NPCs. A promising binding site was identified between miR-25 and SUMO2 using bioinformatics analysis (targetscan.org/vert_72/; Fig. 3A). More specifically, a binding site of miR-25 was identified in the SUMO2 3'-UTR (Fig. 3A). To verify the direct binding of miR-25 to SUMO2, a luciferase reporter assay was performed. The results demonstrated that NPC transfection with miR-25 mimic significantly attenuated the luciferase activity of the WT, but not that of the Mut SUMO2 3'-UTR, indicating that miR-25 could directly target SUMO2 (Fig. 3B). In addition, to verify the direct interaction between miR-25 and

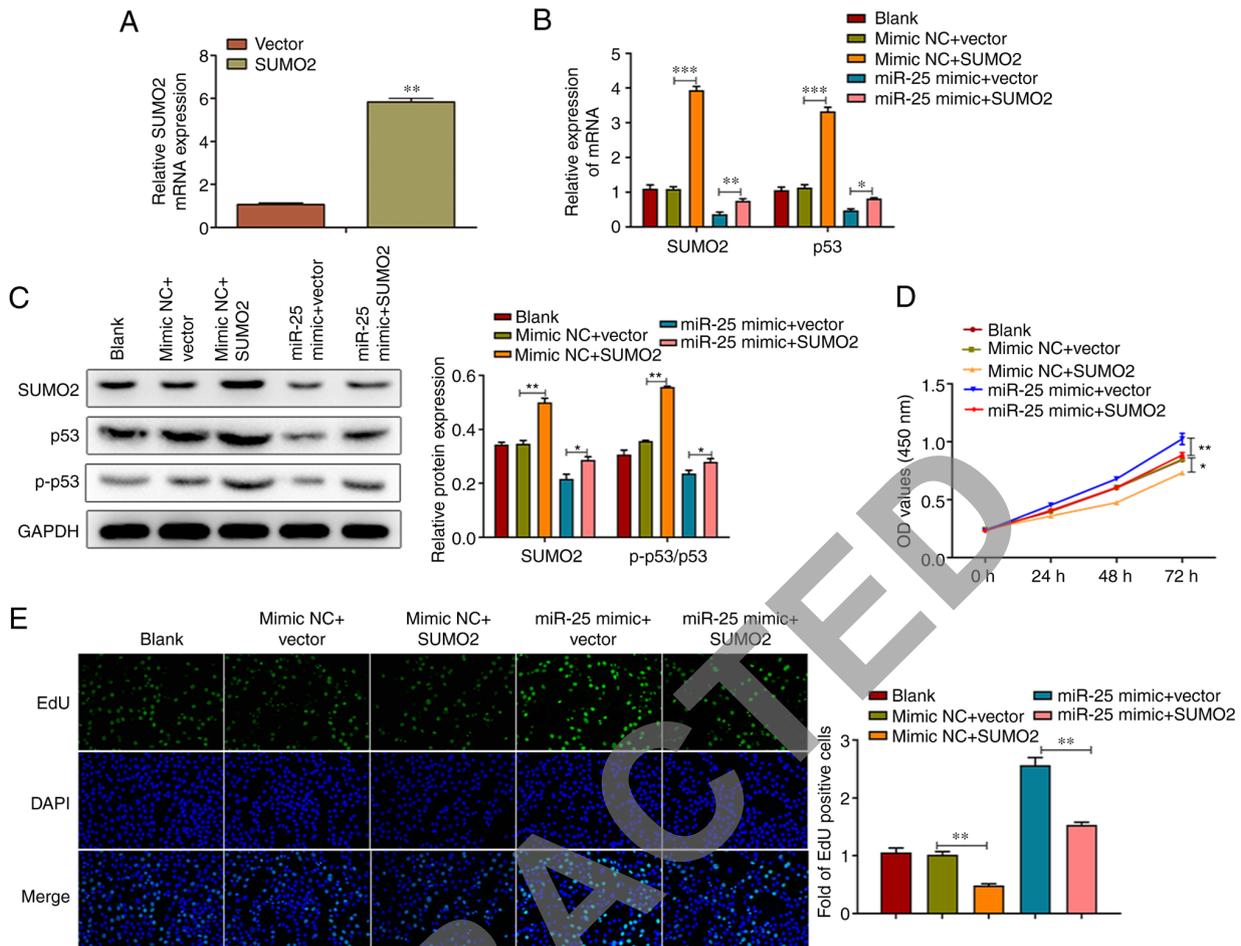


Figure 4. miR-25 promotes human NPC proliferation via targeting SUMO2 through the p53 signaling pathway. (A) Transfection efficiency was detected following cell transfection with SUMO2 overexpression vector. $^{**}P<0.01$ vs. vector. (B and C) mRNA and protein expression levels of SUMO2, p53 and p-p53 were assessed using reverse transcription-quantitative PCR and western blot analysis, respectively. (D and E) Cell Counting Kit-8 and EdU assays were carried out to evaluate human NPC proliferation in cells overexpressing both miR-25 and SUMO2. Data are expressed as the mean \pm SD (n=3). Magnification, $\times 200$. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$. NPCs, nucleus pulposus cells; SUMO2, small ubiquitin-related modifier 2; miR, microRNA; p-, phosphorylated; NC, negative control; EdU, 5-ethynyl-2'-deoxyuridine.

SUMO2, RNA pull-down assay with biotinylated miRNA was carried out in NPCs transfected with bio-miR-25 or bio-NC. Following transfection for 48 h, SUMO2 mRNA was notably enriched in the bio-miR-25 group compared with the bio-NC group (Fig. 3C). As shown in Fig. 3D and E, overexpression of miR-25 significantly reduced the mRNA and protein levels of SUMO2 in NPCs, whereas NPC transfection with miR-25 inhibitor had the opposite effect.

miR-25 acts via targeting SUMO2 through the p53 signaling pathway in human NPCs. To investigate whether the effects of miR-25 in human NPCs were mediated via targeting SUMO2, human NPCs were transfected with SUMO2 to overexpress SUMO2 expression, following which, the transfection efficiency was detected (Fig. 4A). Moreover, rescue experiments were performed. Therefore, SUMO2 overexpression partially abrogated the miR-25 overexpression-mediated upregulation of SUMO2, p53 and p-p53 (Fig. 4B and C). Furthermore, CCK-8 and EdU assays revealed that the overexpression of SUMO2 significantly reversed the miR-25 overexpression-induced NPC proliferation (Fig. 4D and E). Additionally, flow cytometry results showed that the miR-25-mediated inhibition of

apoptosis in NPCs was attenuated by SUMO2 overexpression (Fig. 5A). In addition, western blot analysis demonstrated that the overexpression of SUMO2 could prevent the miR-25 mimic-mediated Bax downregulation and Bcl-2 upregulation in human NPCs (Fig. 5B).

Discussion

Degeneration of the intervertebral disc is accompanied by a decrease in the cell count and synthesis of the extracellular matrix (17). The role of miRNAs in IVDD has attracted extensive attention in recent years. It has been reported that several miRNAs are differentially expressed in IVDD, including miR-222, miR-589, miR-574-3p, miR-199a-5p and miR-483-5p (24,25). Consistent with a previous study (17), the expression of miR-25 was significantly decreased in NP tissues of patients with IVDD in the present study.

NP cell apoptosis is an important cause of IVDD (26). Thus, the present study analyzed the effect of miR-25 on NPC cell proliferation and apoptosis. The functional analysis demonstrated that miR-25 overexpression increased the proliferation of human NPCs and suppressed apoptosis, while

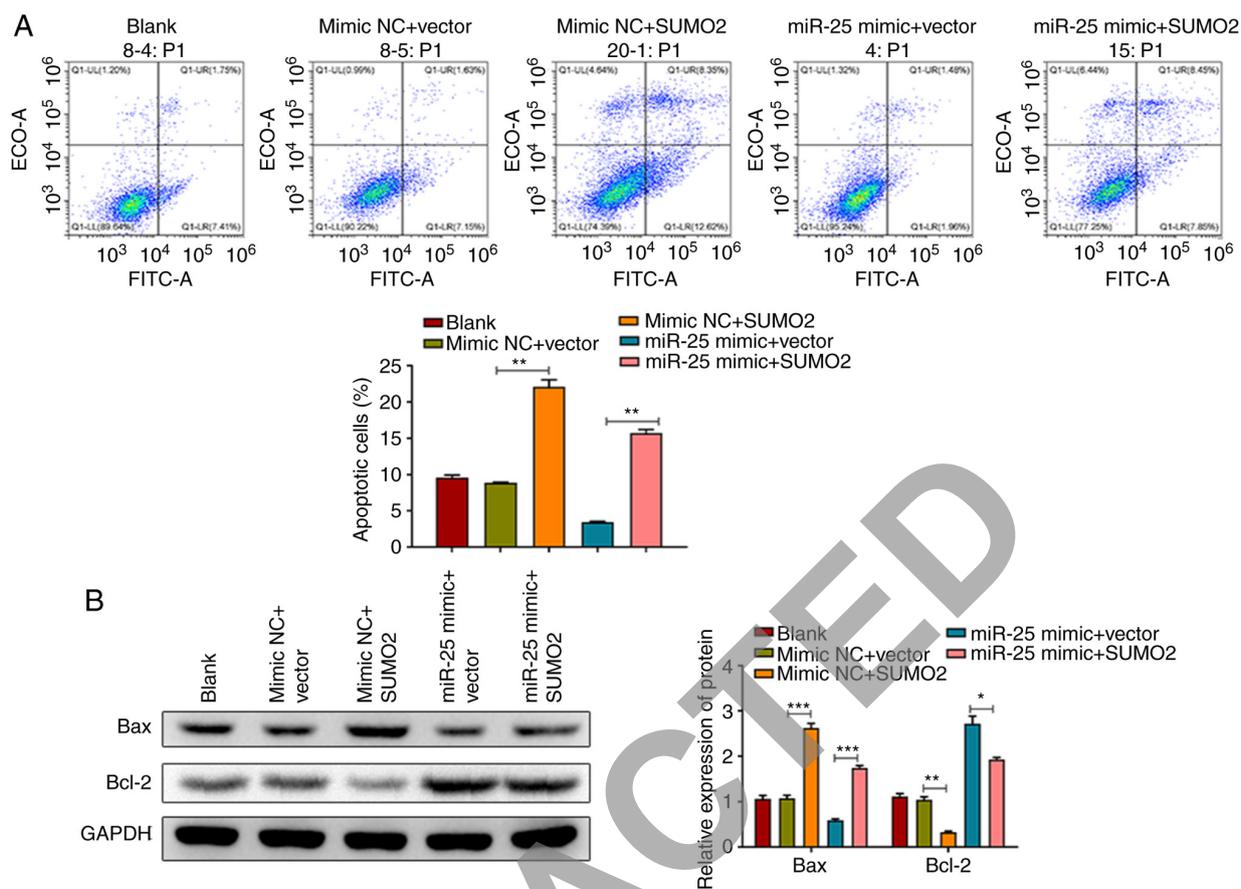


Figure 5. miR-25 attenuates the apoptosis of human NPCs via targeting SUMO2. (A) Cell apoptosis rate was determined by flow cytometry. (B) Protein expression levels of Bax and Bcl-2 in human NPCs overexpressing both miR-25 and SUMO2. Data are expressed as the mean \pm SD (n=3). * P <0.05, ** P <0.01 and *** P <0.001. NPCs, nucleus pulposus cells; SUMO2, small ubiquitin-related modifier 2; miR, microRNA; NC, negative control.

miR-25 knockdown reduced NPC proliferation. These findings suggested that miR-25 downregulation could be implicated in the development of IVDD.

Although the molecular mechanism underlying IVDD has not been fully elucidated, it is speculated that the apoptosis-mediated aberrant loss of NPCs is the pathogenic process underlying IVDD, and several miRNAs play vital roles in this pathogenic process. For example, miR-21, secreted by MSC-derived exosomes, may protect human NPCs against apoptosis via targeting PTEN via the PI3K/AKT signaling pathway (27). Additionally, miR-532 can contribute to the loss of NPCs via the Bcl-9-mediated Wnt/ β -catenin signaling, resulting in IVDD development (28). In addition, another study revealed that miR-222 knockdown could inhibit the apoptosis of human NPCs and inflammation in IVDD via regulating tissue inhibitor of metalloproteinase-3 (29). Exploring the underlying mechanism by which miRNAs regulate the development of IVDD may have important implications for the discovery of novel therapeutic targets.

miRNAs regulate the expression of their target genes via inhibiting the translation or mediating the degradation of their target mRNAs (30,31). In the present study, bioinformatics analysis predicted putative miR-25 binding sites in the 3'-UTR of SUMO2. Consistently, SUMO2 was identified as a direct target of miR-25 using luciferase reporter assay combined with RNA pull-down assays. The regulatory effect of miR-25

on SUMO2 expression was further confirmed by RT-qPCR and western blot analysis. Therefore, it was hypothesized that miR-25 may regulate the proliferation of NPCs in IVDD via SUMO2.

SUMOs belong to a group of ubiquitin-like proteins, which can be covalently connected to some substrate proteins such as I κ B α , c-Jun and p53, to participate in post-translational modification, regulate subcellular localization and protein interactions, and promote proteasome degradation (32). SUMO2 is involved in the regulation of apoptosis-associated signaling pathways, such as the p53, death-associated protein and dynamin-related protein 1 pathways (33-35). As a member of the SUMO superfamily, SUMO2 plays a crucial role in the degradation and apoptosis of NPCs through the activation of the p53 signaling pathway (36-38). In line with previous studies, the results of further experiments demonstrated that SUMO2 overexpression reversed the effects of miR-25 overexpression on NPC proliferation and apoptosis, and the inhibition of p53 phosphorylation.

In conclusion, the findings of the present study suggested that miR-25 may improve NPC proliferation and inhibit apoptosis, partly via inhibiting the SUMO2-mediated p53 signaling pathway. Therefore, strategies upregulating miR-25 expression may be considered as effective therapeutic approaches to IVDD. A future follow-up study is necessary to clarify and further investigate the other underlying mechanisms of miR-25 on IVDD progression.

Acknowledgements

Not applicable.

Funding

This study was supported by the Science and Technology Project of Hunan Provincial Department of Education (grant no. 19C1619).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CL designed the experiments. CL and JL were the major contributors in writing the manuscript. JL, GT and JW performed the experiments and analyzed data. All authors have read and approved the final manuscript. CL and JL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The current study was approved by the Ethics Committee of the Affiliated Hospital of Xiangnan University (Chenzhou, China). All subjects provided written informed consent prior to enrollment.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Vergroesen PP, Kingma I, Emanuel KS, Hoogendoorn RJ, Welting TJ, van Royen BJ, van Dieën JH and Smit TH: Mechanics and biology in intervertebral disc degeneration: A vicious circle. *Osteoarthritis Cartilage* 23: 1057-1070, 2015.
- Sampara P, Banala RR, Vemuri SK, Av GR and Gpv S: Understanding the molecular biology of intervertebral disc degeneration and potential gene therapy strategies for regeneration: A review. *Gene Ther* 25: 67-82, 2018.
- Cui S and Zhang L: circ_001653 silencing promotes the proliferation and ECM synthesis of NPCs in IDD by downregulating miR-486-3p-mediated CEMIP. *Mol Ther Nucleic Acids* 20: 385-399, 2020.
- Zhan S, Wang K, Xiang Q, Song Y, Li S, Liang H, Luo R, Wang B, Liao Z, Zhang Y and Yang C: lncRNA HOTAIR upregulates autophagy to promote apoptosis and senescence of nucleus pulposus cells. *J Cell Physiol* 235: 2195-2208, 2020.
- Sheppard JJ, Malandraki GA, Pifer P, Cuff J, Troche M, Hemsley B, Balandin S, Mishra A and Hochman R: Validation of the choking risk assessment and pneumonia risk assessment for adults with intellectual and developmental disability (IDD). *Res Dev Disabil* 69: 61-76, 2017.
- Li Z, Li X, Chen C, Li S, Shen J, Tse G, Chan MTV and Wu WKK: Long non-coding RNAs in nucleus pulposus cell function and intervertebral disc degeneration. *Cell Prolif* 51: e12483, 2018.
- Zhu J, Zhang X, Gao W, Hu H, Wang X and Hao D: lncRNA/circRNA-miRNA-mRNA ceRNA network in lumbar intervertebral disc degeneration. *Mol Med Rep* 20: 3160-3174, 2019.
- Kim DY and Sung JH: Regulatory role of microRNAs in the proliferation and differentiation of adipose-derived stem cells. *Histol Histopathol* 32: 1-10, 2017.
- Zhou X, Chen L, Grad S, Alini M, Pan H, Yang D, Zhen W, Li Z, Huang S and Peng S: The roles and perspectives of microRNAs as biomarkers for intervertebral disc degeneration. *J Tissue Eng Regen Med* 11: 3481-3487, 2017.
- Zhang CY, Chen CY, Wen HX, Song ZF and Hu PP: miR-182-5p enhances cisplatin resistance in epithelial ovarian cancer by downregulating GRB2. *Eur J Gynaecol Oncol* 42: 353-359, 2021.
- Yang B, Li S, Zhu J, Huang S, Zhang A, Jia Z, Ding G and Zhang Y: miR-214 protects against uric acid-induced endothelial cell apoptosis. *Front Med (Lausanne)* 7: 411, 2020.
- Wu L, Cheng S, Meng Y and Huang Y: miR-194 regulates cisplatin resistance in colorectal cancer cells through targeting yes-associated protein. *J Biomater Tissue Eng* 10: 157-162, 2020.
- Zhou J and Zhan Y: microRNA-221 (Mir-221) influences ovarian cancer cell proliferation and apoptosis through regulating suppressors of cytokine signaling 3-janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) pathway. *J Biomater Tissue Eng* 10: 889-894, 2020.
- Chen H, Pan H, Qian Y, Zhou W and Liu X: MiR-25-3p promotes the proliferation of triple negative breast cancer by targeting BTG2. *Mol Cancer* 17: 4, 2018.
- Wan W, Wan W, Long Y, Li Q, Jin X, Wan G, Zhang F, Lv Y, Zheng G, Li Z and Zhu Y: MiR-25-3p promotes malignant phenotypes of retinoblastoma by regulating PTEN/Akt pathway. *Biomed Pharmacother* 118: 109111, 2019.
- Liu B and Sun X: miR-25 promotes invasion of human non-small cell lung cancer via CDH1. *Bioengineered* 10: 271-281, 2019.
- Zhao B, Yu Q, Li H, Guo X and He X: Characterization of microRNA expression profiles in patients with intervertebral disc degeneration. *Int J Mol Med* 33: 43-50, 2014.
- Liang Q, Deng H, Li X, Wu X, Tang Q, Chang TH, Peng H, Rauscher FJ III, Ozato K and Zhu F: Tripartite motif-containing protein 28 is a small ubiquitin-related modifier E3 ligase and negative regulator of IFN regulatory factor 7. *J Immunol* 187: 4754-4763, 2011.
- Agbor TA, Cheong A, Comerford KM, Scholz CC, Bruning U, Clarke A, Cummins EP, Cagney G and Taylor CT: Small ubiquitin-related modifier (SUMO)-1 promotes glycolysis in hypoxia. *J Biol Chem* 286: 4718-4726, 2011.
- Kim YR, Jacobs JS, Li Q, Gaddam RR, Vikram A, Liu J, Kassar M, Irani K and Kumar S: SUMO2 regulates vascular endothelial function and oxidative stress in mice. *Am J Physiol Heart Circ Physiol* 317: H1292-H1300, 2019.
- Oh CH, Kim DY, Ji GY, Kim YJ, Yoon SH, Hyun D, Kim EY, Park H and Park HC: Cervical arthroplasty for moderate to severe disc degeneration: Clinical and radiological assessments after a minimum follow-up of 18 months-pfirrmann grade and cervical arthroplasty 55. *Yonsei Med J* 55: 1072-1079, 2014.
- Zhang B, Guo W, Sun C, Duan HQ, Yu BB, Mu K, Guan YY, Li Y, Liu S, Liu Y, *et al*: Dysregulated MiR-3150a-3p promotes lumbar intervertebral disc degeneration by targeting aggrecan. *Cell Physiol Biochem* 45: 2506-2515, 2018.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Sherafatian M, Abdollahpour HR, Ghaffaripasand F, Yaghmaei S, Azadegan M and Heidari M: MicroRNA expression profiles, target genes, and pathways in intervertebral disk degeneration: A meta-analysis of 3 microarray studies. *World Neurosurg* 126: 389-397, 2019.
- Hu P, Feng B, Wang G, Ning B and Jia T: Microarray based analysis of gene regulation by microRNA in intervertebral disc degeneration. *Mol Med Rep* 12: 4925-4930, 2015.
- Zhang Z, Huo Y, Zhou Z, Zhang P and Hu J: Role of lncRNA PART1 in intervertebral disc degeneration and associated underlying mechanism. *Exp Ther Med* 21: 131, 2021.
- Cheng X, Zhang G, Zhang L, Hu Y, Zhang K, Sun X, Zhao C, Li H, Li YM and Zhao J: Mesenchymal stem cells deliver exogenous miR-21 via exosomes to inhibit nucleus pulposus cell apoptosis and reduce intervertebral disc degeneration. *J Cell Mol Med* 22: 261-276, 2018.

28. Sun Z, Jian Y, Fu H and Li B: MiR-532 downregulation of the Wnt/ β -catenin signaling via targeting Bcl-9 and induced human intervertebral disc nucleus pulposus cells apoptosis. *J Pharmacol Sci* 138: 263-270, 2018.
29. Zhang Y, Yang J, Zhou X, Wang N, Li Z, Zhou Y, Feng J, Shen D and Zhao W: Knockdown of miR-222 inhibits inflammation and the apoptosis of LPS-stimulated human intervertebral disc nucleus pulposus cells. *Int J Mol Med* 44: 1357-1365, 2019.
30. Tafrihi M and Hasheminasab E: MiRNAs: Biology, biogenesis, their web-based tools, and databases. *Microna* 8: 4-27, 2019.
31. Liu B, Li J and Cairns MJ: Identifying miRNAs, targets and functions. *Brief Bioinform* 15: 1-19, 2014.
32. Zhao X: SUMO-mediated regulation of nuclear functions and signaling processes. *Mol Cell* 71: 409-418, 2018.
33. Mahmud I and Liao D: DAXX in cancer: Phenomena, processes, mechanisms and regulation. *Nucleic Acids Res* 47: 7734-7752, 2019.
34. Choi SG, Kim H, Jeong EI, Lee HJ, Park S, Lee SY, Lee HJ, Lee SW, Chung CH and Jung YK: SUMO-modified FADD recruits cytosolic Drp1 and caspase-10 to mitochondria for regulated necrosis. *Mol Cell Biol* 37: e00254-16, 2017.
35. Ashikari D, Takayama K, Tanaka T, Suzuki Y, Obinata D, Fujimura T, Urano T, Takahashi S and Inoue S: Androgen induces G3BP2 and SUMO-mediated p53 nuclear export in prostate cancer. *Oncogene* 36: 6272-6281, 2017.
36. Wang F, Cai F, Shi R, Wei JN and Wu XT: Hypoxia regulates sumoylation pathways in intervertebral disc cells: Implications for hypoxic adaptations. *Osteoarthritis Cartilage* 24: 1113-1124, 2016.
37. Jin LZ, Lu JS and Gao JW: Silencing SUMO2 promotes protection against degradation and apoptosis of nucleus pulposus cells through p53 signaling pathway in intervertebral disc degeneration. *Biosci Rep* 38: BSR20171523, 2018.
38. Yu S, Galeffi F, Rodriguiz RM, Wang Z, Shen Y, Lyu J, Li R, Bernstock JD, Johnson KR, Liu S, *et al*: Small ubiquitin-like modifier 2 (SUMO2) is critical for memory processes in mice. *FASEB J* 34: 14750-14767, 2020.

RETRACTED