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Hsa_circ_0001946 Ameliorates Mechanical Stress-induced Intervertebral Disk Degeneration Via Targeting miR-432-5p and SOX9

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Study Design. Experimental analysis of circular RNA in intervertebral disk degeneration (IDD).

Objective. This study aimed to explore the roles of hsa_circ_0001946 (circ-CDR1as) in mechanical stress-induced nucleus pulposus cell injury in IDD.

Summary of Background Data. Mechanical stress is an important pathogenic factor for IDD. Excessive compression stress leads to nucleus pulposus (NP) cell apoptosis and extracellular matrix (ECM) degradation and accelerated IDD. Circ-CDR1as is associated with various degenerative conditions, but its role in IDD is not clear. Herein, we explored the roles and mechanisms of circ-CDR1as in IDD in vitro.

Materials and Methods. An in vitro model of IDD was constructed by treating NP cells with 1.0 MPa compression stress. Quantitative real-time polymerase chain reaction assay was used for detecting the expression of circ-CDR1as and miR-432-5p. Immunofluorescent analysis was performed for MMP13 detection. Western blot assay was performed for detecting apoptosis and ECM-related protein expression. Flow cytometry analysis was used for cell apoptosis analysis. The dual-luciferase reporter was used to analyze the interaction between miR-432-5p and circ-CDR1as or SOX9. Differences in means between groups were evaluated using the Student *t* test or one-way analysis of variance.

Results. In compression-treated human NP cells, we found that circ-CDR1as was significantly downregulated. Functional experiments showed that circ-CDR1as overexpression reduced the compression-induced apoptosis and ECM degradation in NP cells. Further research indicated that circ-CDR1as could act as a molecular sponge for miR-432-5p, a miRNA that enhanced compression-induced damage of NP cells by inhibiting the expression of SOX9. The luciferase reporter experiments also showed that the mutual dialogue between circ-CDR1as and miR-432-5p regulated the expression of SOX9.

Conclusions. Circ-CDR1as binds to miR-432-5p and plays a protective role in mitigating compression-induced NP cell apoptosis and ECM degradation by targeting SOX9. Circ-CDR1as may provide a novel therapeutic target for the clinical management of IDD in the future.

Key Words: nucleus pulposus cell, intervertebral disk degeneration, mechanical stress, circ-CDR1as, microRNA sponge

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Low back pain (LBP) is one of the most common health problems, with a serious burden to the global economy and society.[1] About 80% of people will suffer from LBP in their lifetime.[2] Intervertebral disk degeneration (IDD) is an important cause of LBP. Currently, clinical treatments for IDD are limited to relieve pain and control symptoms, and more effective therapeutic strategies involving the pathologic mechanisms of IDD remain underexplored. The structure of intervertebral disk (IVD) mainly comprises nucleus pulposus (NP), annulus fibrosus, and cartilage endplates.[3] Among the main components of IVD, NP is the structural and functional center, and the extracellular matrix (ECM) secreted by NP cells is responsible for maintaining the biomechanical stability of the spine.[4]

The pathogenesis of IDD is closely associated with mechanical overload.[5] Previous studies have shown that

excessive compression increased apoptosis and reduced ECM components of NP cells, and thus contributed to IDD development.[6,7] NP cell apoptosis is accompanied by the upregulation of apoptosis-inducing protein Bax and the downregulation of antiapoptotic protein Bcl-2.[8] Matrix metalloproteinase 13 (MMP13) is an important matrix-decomposing enzyme, which degrades the main components of ECM and causes dehydration and absorption of NP.[9] Sry-related HMG box-containing gene 9 (SOX9) is a known regulator of chondrogenesis.[10] SOX9 promotes the expression of ECM components in cartilage.[11] NP cells have morphologic and biochemical similarity to chondrocytes, and SOX9 is also an important protector for NP cells. SOX9 promotes the expression of collagen II and aggrecan, and inhibits the expression of MMP13, thereby maintaining the ECM homeostasis in NP.[12–14] Tsingas *et al*[15] found that deletion of SOX9 in mice accelerated NP cell apoptosis and matrix remodeling, and led to severe IDD. It has been reported that mechanical stress can inhibit the expression of SOX9 in NP cells.[16] However, the regulatory mechanism of noncoding RNAs that specifically regulate SOX9 in NP cells under compression is still unclear.

Circular RNA (CircRNA) is a conserved and widely expressed noncoding RNA, formed by a covalently closed continuous loop of RNA. It usually exhibits tissue-specific or development-specific expression.[17] Different from linear RNA, circRNA has more stable characteristics, which is advantageous in clinical applications as biomarkers or treatments.[18] The circRNA has multiple microRNA (miRNA) response elements that absorb miRNA like a sponge and indirectly regulate the expression of targeted genes.[19] The interaction between circRNA and miRNA shows the potential of circRNAs to regulate various degenerative conditions. Indeed, studies have reported different circRNAs that can promote, or inhibit, IDD.[20] A previous study revealed that circRNA_0000253 can downregulate Sirtuin1 and accelerate IDD by adsorbing miRNA-141-5.[21] Our previous study found that circRNA-CIDN acts as a sponge for miR-34a-5p, thereby reversing SIRT1 inhibition in NP cells.[6] However, there are still a large number of circRNAs that have not been effectively studied in IDD. Several works have shown that hsa_circ_0001946 (circ-CDR1as) acts as a molecular sponge for a variety of miRNAs and is responsible for the occurrence and development of many degenerative conditions.[22–24] However, the role of circ-CDR1as in IDD is still unclear. In the current study, we evaluated the role of circ-CDR1as in compression-induced NP cell apoptosis and ECM degradation. We hypothesized that circ-CDR1as could increase SOX9 expression by inhibiting miR-432-5p in NP cells, and play a protective role in IDD induced by compression.

MATERIALS AND METHODS

NP Tissues Collection and Cell Culture

A total of six IVD tissue specimens were taken from patients with idiopathic scoliosis undergoing spinal surgery (three males and three females, 20–27 yr, with an average of 22.5 yr). The degree of IVD degeneration was assessed by the Pfirrmann MRI grading system, and the Pfirrmann grades of these specimens were generally I or II. All procedures obtained consents from each informed subject. This study was guided by the Clinical Research Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology, and all methods were used in strict accordance with the approved guidelines. For the NP cells isolation and culture, detailed treatments were performed as described previously.[6]

Western Blotting

SDS-PAGE of 10% to 12% (Bio-Rad) was utilized to separate the proteins of equal quality from each sample, which were later transferred to the PVDF membrane (Millipore). The sample was incubated with the specific primary antibody overnight at 4°C and then incubated with the secondary antibody. Primary antibodies against the following proteins were used: glyceraldehyde 3-phosphate dehydrogenase (GAPDH, #5174; CST), Bax (ab32503; Abcam), Bcl-2 (ab196495; Abcam), collagen II (ab34712; Abcam), aggrecan (13880-1-AP; Proteintech), MMP13 (ab39012; Abcam), SOX9 (ab185966; Abcam).

Immunofluorescent Analysis

The immunofluorescent analysis was performed as described before.[3] In short, the NP cells were first fixed with 4% paraformaldehyde and then infiltrated with 0.2% Triton X-100 phosphate-buffered saline (PBS). The slides were then washed with PBS and blocked with 2% bovine serum albumin-PBS at 37°C and were incubated with the MMP13 (1:100) (18165-1-AP; Proteintech) primary antibody overnight at 4°C. After two washes, the slides were treated with a secondary antibody (Boster) at room temperature for 2 hours. Nuclei were stained with DAPI (Beyotime). A fluorescence microscope (Olympus IX71) was used for imaging.

RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR) Assay

Trizol reagent (Invitrogen) was used to extract total RNA from treated cells as previously described.[25] The purity and concentration of RNA were measured with a micro-spectrophotometer. The RNA was then reverse-transcribed utilizing a Transcriptor First Strand cDNA Synthesis Kit (Takara Biotechnology,). Polymerase chain reaction amplification was performed with AmpliTaq DNA Polymerase (Life Technologies). Real-time (RT) qPCR primers are as follows (5′–3′): hsa-miR-432-5p, loop primer GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACT AAC CGAT, F primer TGC GCT CTT GGA GTA GGT CATTG. Homo circ-CDR1as, forward: GTG CTG ATC TTC TGA CATTC, reverse: AGA CCT TGA GAT TAT TGGAA. Divergent homo circ-CDR1as, forward: TCC ATC AAC TGG CTC AAT ATCC, divergent homo circ-CDR1as, reverse: GGATTGTCTG-GAAGATGTGGAT. Homo GAPDH, forward: GTC TTC

ACC ACC ATG GAGAA, reverse: TAA GCA GTT GGT GGT GCAG. U6, forward: CGC TTC GGC AGC ACA TATAC, reverse: AAA TAT GGA ACG CTT CACGA. All circRNA and mRNA expression levels were normalized to GAPDH, and the expression level of miRNA was normalized to U6.

Flow Cytometry Analysis

NP cells with different treatment were collected, and the Annexin V-APC/7-AAD dual staining (KeyGen Biotech) was used to detect the level of cell apoptosis. After twice washing with PBS, the NP cells were suspended in the binding buffer and then incubated with Annexin V-APC and 7-AAD for 15 minutes at room temperature. The entire incubation process was protected from light. A FACS Calibur flow cytometer (BD Biosciences) was used to detect the apoptotic degree of the cell samples.

Cell Transfection

The siRNA and overexpression vector for circ-CDR1as were synthesized by Obio Technology. The siRNA target sequence on circ-CDR1as was 5′-TATCCAGGGTTTC CGATGG-3′. The hsa-miR-432-5p mimics, hsa-miR-432-5p inhibitor, and the negative controls were obtained from GenePharma. The siRNA targeting SOX9 (si-SOX9) and the negative control were constructed by Ribo-bio. And the target sequence on SOX9 was 5′-CGCTCAC AGTACGACTACA-3′. Lipofectamine 3000 was used for transfection when the cultured NP cells grew to 80% confluence. The transfection efficiency was verified at 24 hours after transfection, followed by subsequent cell treatments.

Statistical Analysis

At least three independent experiments were performed and the data were presented as the mean ± SD. GraphPad Prism 7 were used for statistical analyses. Differences in means between groups were evaluated using the Student *t* test or one-way analysis of variance. Only P-value < 0.05 was considered statistically significant.

RESULTS

Effects of circ-CDR1as on Compression-induced NP Cell Apoptosis and ECM Degradation

Under the given pressure stimulus of 1.0 MP for 36 hours,[6] the circ-CDR1as expression level was significantly downregulated (Figure S6A, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). The Sanger sequence analysis verified the circular structure of circ-CDR1as (Figure S6B, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). To determine the effect of circ-CDR1as on compression-induced apoptosis and ECM degradation in NP cells, we overexpressed or downregulated circ-CDR1as and then assayed the expression of related proteins under compression. The effect of interference or overexpression treatment on the expression of circ-CDR1as in NP cells was verified by RT-qPCR (Figure 1A, B). Compared with the control group, knockdown of circ-CDR1as can significantly reduce the ECM proteins of NP and increase the ratio of cell apoptotic indicators, Bax/Bcl-2 (Figure 1C). Correspondingly, overexpression of circ-CDR1as can rescue the ECM degradation and NP cell apoptosis induced by compression (Figure 1D). MMP13 is an important ECM-degrading enzyme in the disk.[26] Fluorescence results showed that the expression of MMP13 was enhanced by the siRNA of circ-CDR1as (Figure S1A, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Whereas overexpression of circ-CDR1as significantly decreased the expression of MMP13, suggesting the potential role of circ-CDR1as in resisting ECM degradation (Figure S1B, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). In addition, the flow cytometry results of apoptosis also showed that overexpression of circ-CDR1as could significantly inhibit the apoptosis rate of NP cells, while circ-CDR1as silence amplified the apoptosis rate (Figure S1C, D, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). The above results indicated that circ-CDR1as played an important role in mitigating compression-induced apoptosis and matrix degradation in NP cells.

Circ-CDR1as Functioned in the Human NP Cells Under Compression Via Targeting miR-432-5p

Considering that circ-CDR1as plays a protective role in various degenerative diseases through its characteristics as a molecular sponge of several miRNAs, we speculated that the protective role of circ-CDR1as against compression-induced NP cell damage may involve a similar mechanism. To find miRNAs that interact with circ-CDR1as, we used the CircRNA Interactome online tool and speculated that miR-432-5p might be a potential downstream target. We predicted the potential binding sites between circ-CDR1as and miR-432-5p (Figure S6C, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Next, we aimed to verify the possibility of miR-432-5p as a downstream target of circ-CDR1as. First, we explored the effects of cotransfection of miR-432-5p mimics and circ-CDR1as on NP cells, and the results showed that miR-432-5p mimics significantly offset the protective effect of circ-CDR1as on NP cells. For the MMP13 expression, miR-432-5p mimics reversed the inhibitory effect of circ-CDR1as (Figure 2). In the circ-CDR1as transfection group, the expression of aggrecan and collagen II was upregulated, but this was significantly suppressed by cotransfection with miR-432-5p mimics (Figure S2A, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). In addition, circ-CDR1as alleviated compression-induced apoptosis of NP cells, while miR-432-5p mimics almost completely offset this protective effect, which was reflected by the inversion of the Bax/Bcl-2 ratio and the flow cytometry results (Figure S2A, B, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). To further investigate whether circ-CDR1as and miR-432-5p directly interact, we conducted luciferase reporter experiments. The luciferase reporter vectors of

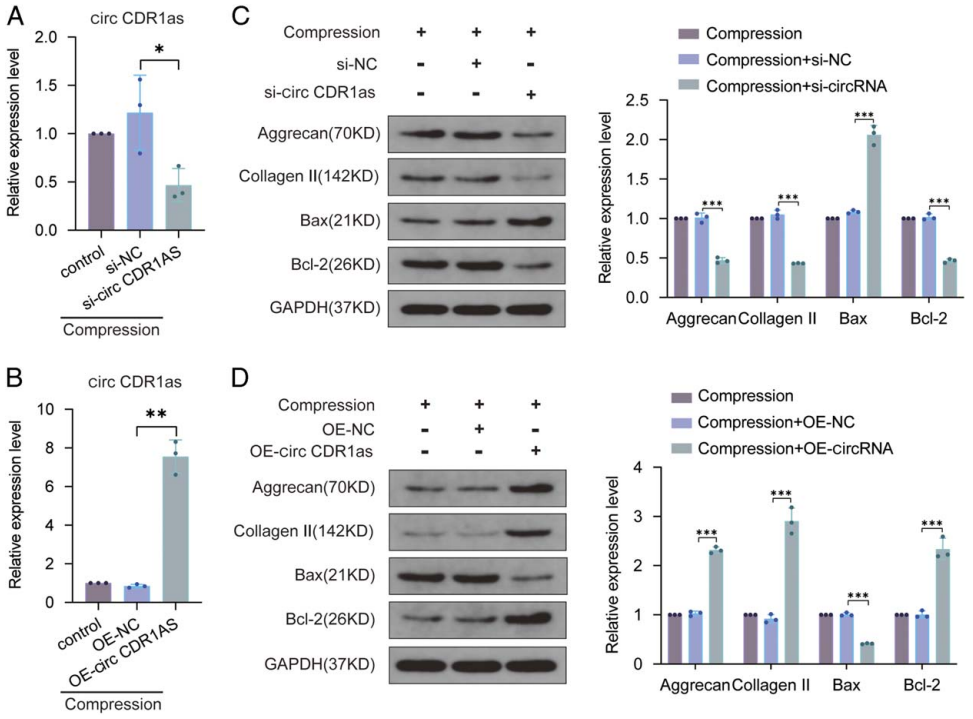

Figure 1. Effects of circ-CDR1as on compression-induced NP cell apoptosis and extracellular matrix degradation. A and B, The quantitative real-time polymerase chain reaction analysis was used to determine the expression level of circ-CDR1as in human NP cells treated with circ-CDR1as siRNA (si) or circ-CDR1as OE vector. C, The western blot analysis was applied to detect the protein levels of aggrecan, collagen II, Bax, and Bcl-2 in human NP cells transfected with circ-CDR1as siRNA under compression treatment. GAPDH served as an internal control. D, The protein levels of aggrecan, collagen II, Bax, and Bcl-2 in human NP cells transfected with circ-CDR1as OE vector under compression treatment. GAPDH served as an internal control. Data were shown as means with error bars representing SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3. circRNA indicates circular RNA, GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NP, nucleus pulposus; OE, overexpression.

mutant (MUT) or wild-type (WT) circ-CDR1AS were constructed and cotransfected to the HEK293T cells with miR-432-5p mimics. The relative luciferase activity was determined as the ratio of firefly luciferase/Renilla luciferase, which is lower in the WT-circ-CDR1AS group (Figure S6D, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Taken together, the above results suggested that circ-CDR1as played a protective role against compression-induced damage in NP cells by inhibiting miR-432-5p.

MiR-432-5p Facilitated Compression-induced NP Cell Apoptosis and ECM Degradation

To verify the effect of miR-432-5p inhibitor or mimics, RT-qPCR analysis were used to detect the miR-432-5p expression levels (Figure 3A, B). Western blot analysis showed that the miR-432-5p inhibitor inhibited the expression of Bax and promoted the expression of Bcl-2. Meanwhile, miR-432-5p inhibitor significantly increased the expression levels of aggrecan and collagen II (Figure 3C). However, miR-432-5p mimics had the opposite effects (Figure 3D). The fluorescence semi-quantitative analysis showed that the expression level of MMP13 was significantly enhanced by miR-432-5p mimics, but decreased by miR-432-5p inhibitors (Figure S3A, B, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Apoptosis flow cytometry results indicated that miR-432-5p mimics and inhibitors respectively play a positive and negative role in regulating the apoptosis level of NP cells under mechanical compression (Figure S3C, D, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Taken together, miR-432-5p plays an important role in facilitating compression-induced ECM degradation and apoptosis in NP cells.

SOX9 Was the Direct Target Gene for miR-432-5p in Human NP Cells

It is known that SOX9 plays an important role in regulating the ECM homeostasis and NP cell apoptosis in IVD.[15] A complementary sequence to the miR-432-5p seed region was found in SOX9 using the TargetScan online tool (Figure S6E, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). To determine whether SOX9 is a downstream target that interacts with miR-432-5p, we performed interaction validation with a luciferase reporter assay. A luciferase reporter vector with the WT-SOX9 or MUT-SOX9 in the 3′-UTR was constructed and the results showed that miR-432-5p mimics obviously inhibited the luciferase activity of the WT-SOX9, whereas no significant change in the luciferase activity was detected in the MUT-SOX9 (Figure S6F, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Next, we verified the changes in the expression level of SOX9 under the action of miR-432-5p, and found that miR-432-5p inhibitor can significantly increase the expression of SOX9, while specific SOX9

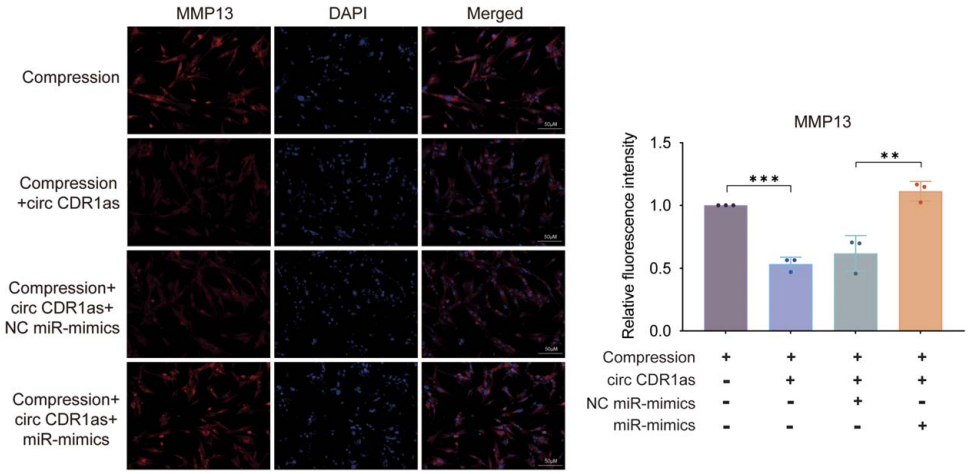

Figure 2. Circ-CDR1as functioned in the human nucleus pulposus cells under compression via targeting miR-432-5p. The human nucleus pulposus cells under compression were pretreated with circ-CDR1as overexpression vector, or circ-CDR1as overexpression with NC mimics, or circ-CDR1as overexpression with miR-432-5p mimics. The MMP13 protein level in the nucleus pulposus cells from each group was determined by immunofluorescence staining, with relative intensity of fluorescence quantified. Original magnification: ×200. Data were presented as means with error bars representing SD. $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3.

siRNA markedly reduced the expression of SOX9 (Figure S4A, B, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Flow cytometry results showed that transfection of si-SOX9 offset the protective effects of miR-432-5p inhibitors on NP cells (Figure S4C, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). The specific knockdown of SOX9 also reversed the effects of miR-432-5p inhibitors on the expression of aggrecan and collagen II, as well as the Bax/Bcl-2 ratio (Figure S4D, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). Immunofluorescence analysis of MMP13 suggested that si-SOX9 reversed the anti-degeneration effect of miR-432-5p inhibitors, indicating that SOX9 serves as the target of miR-432-5p (Figure 4). In summary, these results

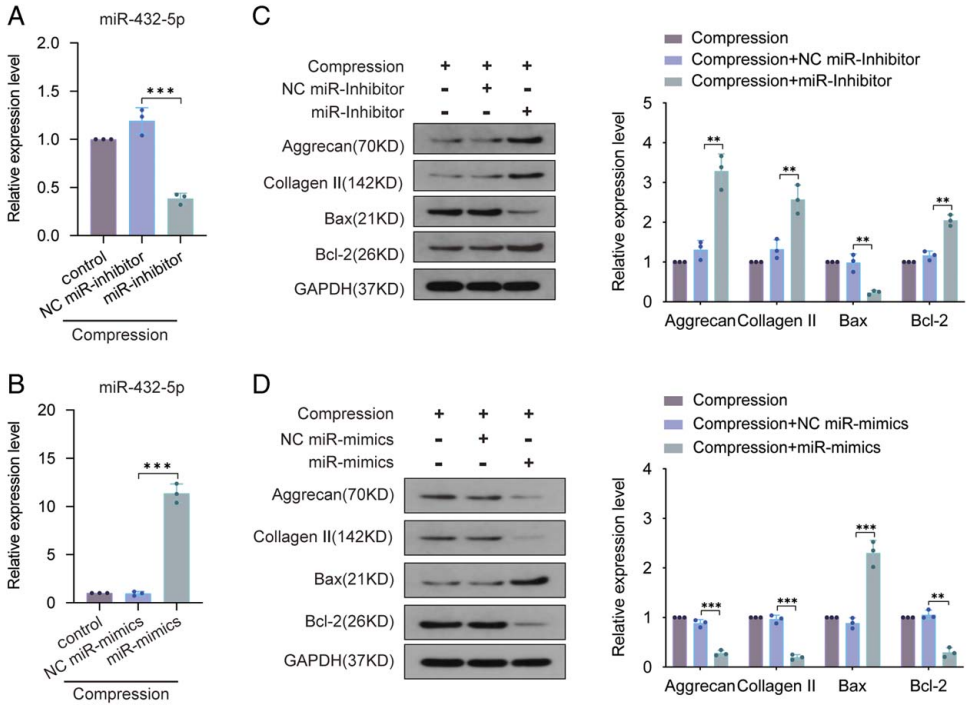

Figure 3. MiR-432-5p facilitated compression-induced NP cell apoptosis and extracellular matrix degradation. A and B, The human NP cells were treated with miR-432-5p inhibitor or miR-432-5p mimics, and the relative expression levels of miR-432-5p were validated by quantitative real-time polymerase chain reaction analysis. C and D, The protein levels of aggrecan, collagen II, Bax, and Bcl-2 in human NP cells treated with miR-432-5p inhibitor or miR-432-5p mimics under compression. GAPDH served as an internal control. Data were presented as means with error bars representing SD. $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3. GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase; NP, nucleus pulposus.

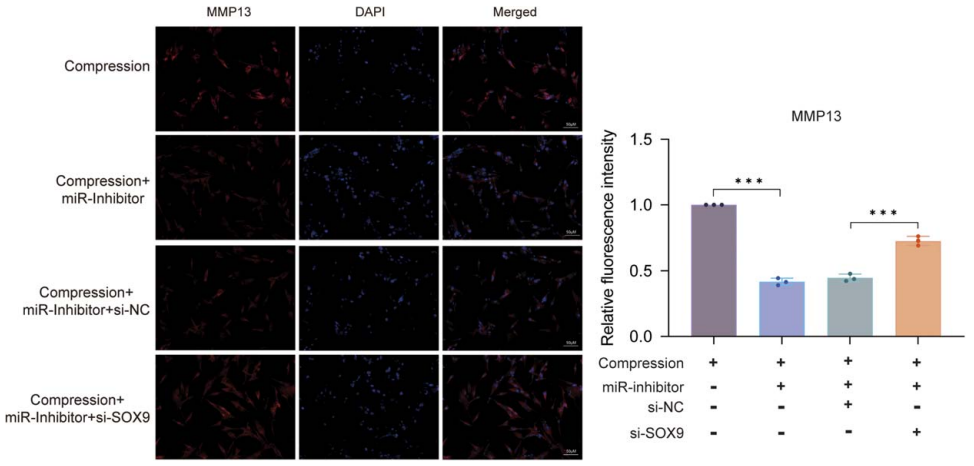

Figure 4. SOX9 was the direct target gene of miR-432-5p in human nucleus pulposus cells. The human nucleus pulposus cells under compression were pretreated with miR-432-5p inhibitor, or miR-432-5p inhibitor with si-NC, or miR-432-5p inhibitor with si-SOX9. The MMP13 protein level in the nucleus pulposus cells from each group was determined by immunofluorescence staining. Original magnification: ×200. Data were shown as means with error bars representing SD. $^{***}P < 0.001$, n = 3.

indicated that the miR-432-5p inhibitor works in resisting compression-induced apoptosis and matrix degradation of NP cells by promoting the expression of SOX9.

Circ-CDR1as Mitigated the NP Cell Apoptosis and ECM Degradation Induced by Compression Via Targeting miR-432-5p and SOX9

Then, we explored whether si-SOX9 cotransfection could interfere with the protective effect of circ-CDR1as on NP cells. The immunofluorescence result of MMP13 showed that the specific interference targeting SOX9 almost completely offset the anti-degradation effect of circ-CDR1as on ECM (Figure 5). The transfection of circ-CDR1as rescued the compression-induced downregulation of aggrecan and collagen II, while cotransfection of si-SOX9 reversed this protective effect of circ-CDR1as (Figure S5A, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). In addition, the detection of apoptosis, including the expression of Bax/Bcl-2 and flow cytometry analysis, also showed that si-SOX9 significantly inhibited the antiapoptotic effect of circ-CDR1as on NP cells under compression, which indicated that the antiapoptotic effect of circ-CDR1as on NP cells is inseparable from its promotion of SOX9 expression (Figure S5A, C, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). To examine whether circ-CDR1as indirectly regulates SOX9 through miR-432-5p, we transfected NP cells with circ-CDR1as in the presence or absence of miR-432-5p mimics and then explored the effect of miR-432-5p mimics on the expression of SOX9 expression. We found that circ-CDR1as transfection significantly increased the expression of SOX9 under compression conditions, while miR-432-5p mimics reversed this effect (Figure S5B, Supplemental Digital Content 1, http://links.lww.com/BRS/C177). The above experiments show that

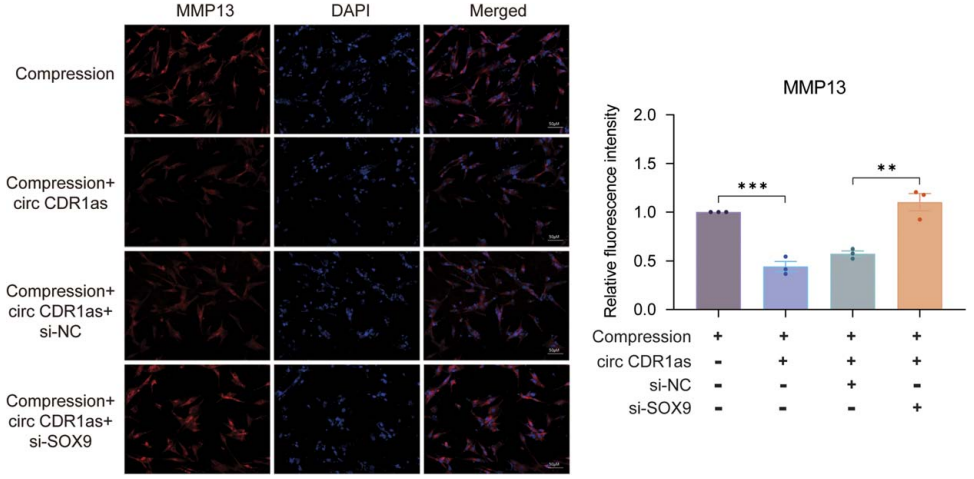

Figure 5. Circ-CDR1as mitigated the extracellular matrix degradation induced by compression via targeting miR-432-5p and SOX9. The human nucleus pulposus cells under compression were pretreated with circ-CDR1as overexpression vector, or circ-CDR1as overexpression with si-NC, or circ-CDR1as overexpression with si-SOX9. The protein expression level of MMP13 in the nucleus pulposus cells from each group was determined by immunofluorescence staining, with relative intensity of fluorescence quantified. Original magnification: ×200. Data were shown as means with error bars representing SD. $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3.

circ-CDR1as targets miR-432-5p and indirectly increases the expression of SOX9 to combat compression-induced apoptosis and matrix degradation in NP cells.

DISCUSSION

The current study explored the posttranscriptional regulatory mechanism of noncoding RNA targeting SOX9 in NP cells under compression stress. Tsingas *et al*[15] discovered that SOX9 deletion in mice causes severe IDD characterized by IVD cell apoptosis and ECM remodeling. Therefore, finding ways to promote SOX9 expression may be a promising direction for IDD treatment. Excessive mechanical load is an acknowledged driver of IDD.[5] Studies have shown that mechanical overload reduced the gene expression of SOX9, collagen II, and aggrecan in NP cells.[16] However, the noncoding RNA-related regulatory mechanism of SOX9 in NP cells under compression stress is still unclear. We searched the potential molecular sponge of SOX9 using bioinformatics predictive analysis, which suggested that miR-432-5p may be a promising one. Furthermore, our studies showed that the expression of SOX9 was inhibited when NP cells were transfected with the miR-432-5p mimic, which mediated SOX9 silencing. The SOX9 inhibition resulted in NP cell apoptosis and matrix catabolism. Therefore, targeting miR-432-5p could be a viable way for altering, or arresting, compression-induced damage in NP cells.

The emergence of the functional roles of circRNAs has provided a new perspective regarding our understanding of cellular physiology and disease pathogenesis. By absorbing disease-related miRNAs, circRNA plays an important regulatory role in various disorders.[27] The bioinformatics analysis indicated that miR-34a-5p is a downstream molecule of circ-CDR1as. In compression-treated NP cells, we found that the circ-CDR1as expression level was significantly downregulated. Notably, either circ-CDR1as overexpression or miR-432-5p inhibitors upregulated SOX9 levels and inhibited apoptosis or ECM degradation in compression-damaged NP cells, while the transient silencing of SOX9 significantly reversed the above effects. The clinical implications of circRNA is an interesting topic because circRNAs are expected to become diagnostic biomarkers and therapeutic targets for degenerative conditions in the future.[28] Previous studies have found that circ-CDR1as is widely involved in the regulation of tumors, arteriosclerosis, Alzheimer disease, Parkinson disease, and other age-related degenerative diseases, which could provide a reference for clinical diagnosis and management.[22–24,29] The current study demonstrates that circ-CDR1as is a positive regulator of SOX9 and may exert an antidegenerative effect in IDD. In the future, circ-CDR1as may serve as a potential mitigative factor in the treatment of IDD.

There are several limitations to our study. First, since circRNA potentially exhibits multiple miRNA or protein binding sites, these were not exhaustively explored. In the future, it will be useful to investigate whether the circ-CDR1as regulatory mechanism for SOX9 involves other miRNAs or RNA-binding proteins. In addition, the present study only explored the molecular mechanisms based on in vitro experiments, and further, in vivo evaluations are required to validate these findings.

CONCLUSIONS

Circ-CDR1as that binds to miR-432-5p plays a protective role in alleviating compression-induced apoptosis and ECM degradation in NP cells by targeting SOX9. This study suggests a potential therapeutic target for the clinical management of IDD in the future.

➢ Key Points

- ❑ Circ-CDR1as was significantly downregulated in compression-treated human NP cells.
- ❑ Circ-CDR1as overexpression mitigated the compression-induced apoptosis and ECM degradation of NP cells.
- ❑ Circ-CDR1as targeted miR-432-5p to regulate the expression of SOX9 in NP cells.
- ❑ Circ-CDR1as may provide a novel therapeutic target for the clinical management of IDD.

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