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Overexpression of miR-150 alleviates mechanical stress-accelerated the apoptosis of chondrocytes *via* targeting GRP94

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Abstract. – OBJECTIVE: A previous study reported that glucose-regulated protein 94 (GRP94) is involved in mechanical stress-induced chondrocyte apoptosis; however, the underlying molecular mechanisms remain unknown. The present study aimed to investigate the post-transcriptional regulatory mechanism of microRNAs (miRs) in mechanical stress-induced chondrocyte apoptosis by targeting GRP94.

MATERIALS AND METHODS: Annexin V-fluorescein isothiocyanate/propidium iodide (PI) staining was conducted to evaluate the apoptosis of chondrocytes. The mRNA and protein expression levels were measured by reverse transcription-quantitative polymerase chain reaction and Western blotting, respectively. The targeted genes were predicted using a bioinformatics tool and further investigated *via* a luciferase reporter assay.

RESULTS: The results demonstrated that cyclic loading led to significant increases in GRP94 expression in chondrocytes. However, the expression levels of miR-150 were regulated. Bioinformatics analysis and a luciferase reporter assay indicated that GRP94 was a direct target of miR-150, as the expression of GRP94 was dysregulated following transfection with miR-150 mimics/inhibitors. In addition, mechanical stress-induced chondrocyte apoptosis was suppressed by transfection with miR-150 mimics, while the promotive effects of miR-150 inhibitors in this process were inhibited by GRP94 overexpression.

CONCLUSIONS: miR-150 upregulation suppresses mechanical stress-induced chondrocyte apoptosis via underlying molecular mechanism mediated, at least partially, *via* the inhibition of GRP94 expression.

Key Words: Mechanical stress, Chondrocyte, Glucose-regulated protein 94, Apoptosis, MiR-150.

Abbreviations

GRP94, glucose-regulated protein 94; miRs, microRNAs; Annexin V-FITC/PI, Annexin V-fluorescein isothiocyanate/propidium iodide; OA, osteoarthritis; MMPs, matrix metallopeptidases; ER, endoplasmic reticulum; ERS, endoplasmic reticulum stress; 3'-UTRs, 3'-untranslated regions; RT-PCR, reverse transcription-quantitative polymerase chain reaction; Tun, tunicamycin; Glu, glucolubrinal.

Introduction

Osteoarthritis (OA) is one of the most common diseases of the elderly and is characterized by the degradation of articular cartilage, thickening of subchondral bone, and the formation of osteophytes[1]. In the early stages of OA, accelerated subchondral bone remodeling may occur in lesions[2]. Generally, aging, vitamin and mineral deficiencies, as well as the incidence of drug abuse, increase the risk of developing OA[3]; however, the pathogenic mechanisms of OA require further investigation. Of note, increasing evidence has revealed that mechanical overload leads to the activation of anabolic processes in chondrocytes involving matrix proteins and matrix-degrading enzymes, such as matrix metallopeptidases[4]. In addition, mechanical stimulation-induced chondrocyte apoptosis has been associated with the progression of OA[5]. Numerous *in vitro* and *in vivo* experiments have suggested that mechanical stimulation contributes to the development of OA by altering specific signaling pathways in chondrocyte apoptosis[5-7].

Glucose-regulated protein-94 (GRP94) is an abundant glycoprotein in the endoplasmic reticulum (ER) and is involved in the maintenance of cell survival by protecting against stresses associated with Ca^{2+} depletion from the ER[8-9]. Previous studies[10-12] revealed that GRP94, as a marker of ER stress (ERS), is activated in mechanical stress-mediated chondrocyte apoptosis and cartilage degeneration; however, the

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post-transcriptional regulatory mechanisms of microRNAs (miRNAs/miRs) targeting GRP94 in mechanical stress-induced chondrocyte apoptosis remain unknown.

MiRNAs are short, noncoding and single-stranded RNAs (18-25 nucleotides), which have been observed to disrupt protein translation via the degradation of transcripts by binding to its 3'-untranslated regions (3'-UTRs); miRNAs serve as a novel class of post-transcriptional regulators that participate in a variety of biological processes[13-15]. It has been demonstrated that numerous miRNAs modulate mechanical stress-induced dysfunction in various cell types, such as chondrocytes[6,16]. For example, miR-365 is upregulated by cyclic loading and contributes to the development of OA[6,17]. MiR-146a expression is increased in response to mechanical pressure-induced human chondrocyte apoptosis *in vitro*[5]. In the present study, the activation of GRP94 signaling in mechanical stress-induced chondrocyte apoptosis was investigated. By utilizing an online prediction algorithm, GRP94 was reported as a potential gene that directly targets miR-150. Furthermore, the function of miR-150 in mechan stress-induced chondrocyte apoptosis via lating GRP94 signaling was determined *in v*

Materials and Me

Cell Culture

Primary human ch dro e from femoral condy d the plateau of knee cartilage a iously de Cells were cultured cco's Mod d Eagle's Medium-F12 Gibco; Thermo Fisher Scienti Waltham, SA) with 5% fetal bovine m (Beyotime te of Biotechnology hen ngsu, China), 2 mM L-glutamine, 1 ml n lin and 100 µg/mL streptomycin we d in a idified incubator (Thermo Fishe tific , Waltham, MA, USA), with % CO_2 air. The present investigation s appro by the Ethics Committee of The Affiliated Hospital of Xi'an Jiaotong Uni- an, China). Written informed consent was obtained from all patients prior to sample collection.

Flow Cytometry for Apoptosis

Human chondrocytes were seeded into three-dimensional collagen sponges as described previously[18] and subjected to differential elongation (0, 5, 10, and 20%), 1 Hz (60 cycles/min) cyclic loading or 10% elongation of various durations (0, 12, 24 or 48 h), 20 min/h, which was monitored by a computer-controlled Bio-Stretch device (Bio-Stretch; ICCT T nologies, Markham, ON, Canada). An A V-fluorescein isothiocyanate (FITC) idium iodide (PI) kit (BD Biosciences, lin Lakes, NJ, USA) was used to in ce 15 min; cell apoptosis was a Led via cytometry (FACScan, BD ienc Fran lin Lakes, NJ, USA) us EL Quest 3.0 software (BD Biosciences, Lak J, USA).

Cell Transfec d Plasmid nstructs

Pre-miR- pre-miR-150, anti-miR-Con, an anti-i were synthesized by Gu u RiboBio Ltd. (Guangzhou, Gua China). Chon ocytes were seeded in ell plates d transfected with pre-miR-C re-miR-1 nti-miR-Con or anti-miR-150 us ofecta ® 2000 (Invitrogen; Thermo Fish nc., Waltham, MA, USA) for 48 h a according to the manufacturer's tocols.

malian expression plasmid (pReceiv- -ERBB3) designed to specially express he full-length open reading frame of human GRP94 without a miR-150 responsive 3'-UTR was purchased from GeneCopoeia, Inc. (Rockville, MD, USA). An empty plasmid served as negative control. The GRP94-overexpression plasmid (vector-GRP94) and control (vector-Con) were transfected into chondrocytes using Lipofectamine 2000 for 48 h at 37°C according to the manufacturer's protocols.

Luciferase Reporter Assay

The sequences of miR-150 were obtained using miRanda (www.microrna.org) and were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, Guangdong, China) The wild-type (WT) and mutant-type (MUT) 3'-UTR of GRP94 were respectively inserted into the multiple cloning sites of the luciferase expressing pMIR-REPORT vector (Ambion; Thermo Fisher Scientific, Inc., Waltham, MA, USA). For the luciferase assay, chondrocytes ($1x10^5$) were seeded into 24-wells and co-transfected with luciferase reporter vectors containing the WT or MUT 3'-UTR of GRP94 (0.5 µg), and mimics or inhibitors sequences of miR-150 (50 nM) using Lipofectamine 2000. The luciferase activity was measured using

a luciferase reporter assay kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocols.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA was isolated using RNAiso (Takara Biotechnology, Ltd., Dalian, Liaoning, China). MiRNA was subsequently reverse-transcribed to cDNA using the TaqMan reverse transcription kit (Applied Biosystems, Foster City, CA, USA; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocols. MiRNA expression was detected using the TaqMan MicroRNA assay (Applied Biosystems, Foster City, CA, USA; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocols. The relative expression levels of miRNA were calculated using the $2^{-\Delta\Delta Cq}$ method[19] and normalized to the internal control U6.

cDNA was synthesized by RT of 2 µg total RNA using Moloney murine leukemia virus reverse transcriptase (Invitrogen, Calsbad, CA, USA; Thermo Fisher Scientific, Inc., Walth MA, USA) according to the manufacturer tocols. RT-qPCR was performed on App Biosystems 7300 Real-Time PCR System (plied Biosystems; Thermo Fisher S ific, In Waltham, MA, USA) with the T iversa PCR Master Mix (Thermo Fi Sci c, Inc., Waltham, MA, USA). T ti

levels of mRNA were calculated using the $2^{-\Delta\Delta Cq}$ method[18] and normalized to the expression of GAPDH. The primers were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The primers were used as shown in Table I.

Western Blotting

Proteins were extracted with radioim e-cipitation assay (RIPA) buffer (ca no. P Beyotime Institute of Biotec gy, Hai Jiangsu, China); protein con ation ere de termined using the Bicinc A BCA) Kit for Protein Determinat BCA1 ; Sigma-Aldrich; Me GaA ma The primary antibody RP94 (cat 93402; dilution: 1:1,00 rchased fro nta Cruz Biotechnolo Cruz, CA, USA). Antibodies agai t cleav ase-3 and cleaved-caspas re purchas m Cell Signaling Tec Inc., (Danvers MA, USA). Subsequ , the mem anes were incubated with an ap riate hor ish peroxidase (HRP)-conjuga condar body (cat. No. sc-516102; dilutio nta Cruz Biotechnology, Inc., (Santa A, USA) at room temperature for and visualized with an enhanced chemilu- e kit (ECL; Thermo Fisher Scientific, altham, MA, USA). Signals were analyzed with Quantity One® software version 4.5 (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Anti-β-actin (cat. No. sc-130065; 1: 2,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used to as the control antibody.

Table I. Primers for R .

Gene	ward prime 3'-3')	Reverse primer (5'-3')
miR-23a	CACAT GGGATTTCC	TGGTGTCGTGGAGTCG
miR-23	GGTGCTCT CTTGG	GCCAAGGTCGTGGTTGCG
miR-	CTCAACTGGT GTGGAGTCGGCAATTC	ACACTCCAGCTGGGTCTCCCAACCCTTGTA
	TGAGCACTGGTA	
2	AGAGGTTCTGTGATACACTC	GGTCCAGTTTTTTTTTTTTTTAGTC
	ATGC GTGCACTACAGAA	GTGCAGGGTCCGAGGT
mi	GC GTGCATCACAGAA	CAGTGCGTGTCGTGGAG
miR-1	ACATTCAACGCTGTC	GTGCAGGGTCCGAGGT
iR-181	CTCCAGCTGGGACTTGGGCACTGAAACA	GTGCAGGGTCCGAGGT
iR-181c	TCTTCAACATTCAACCTGTCG	TATCGTTGTACTCCAGACCAAGAC
1d	CTCATAAACATTCATT GTTGTCGG	CTCATAAACATTCAT TGTTGTCGG
	TCGGCAATCATGATGGGCTCCTC	CTCAACTGGTGTCGTGGAGTC
miR-	CGCGGTGGAATGTAAAGAAG	GTGCAGGGTCCGAGGTATTC
miR-206	ATCCAGTGCGTGTCGTG	TGCTTGGAATGTAAGGAAG
iR-613	GTGAGTGCGTTTCCAAGTGT	TGAGTGGCAAAGAAGGAACAT
miR-223	TCGGCAGGTGTCAGTTTGTCAA	CTCAACTGGTGTCGTGGAGT
miR-425	TCTACCGGTGTGCCCCTGACC CCCAGACA	TCTGAATTCAGCAGGGAAACCCAGGGGCA
U6	CTCGCTTCGGCAGCACATATACT	ACGCTTCACGAATTTGCGTGTC
GRP94	GCTTCGGTCAGGGTATCTTT	AGGCTCTTCTTCCACCTTTG
GAPDH	GCACCGTCAAGCTGAGAAC	TGGTGAAGACGCCAGTGGA

Statistical Analysis

Data were presented as the mean ± standard error of the mean. Statistical analysis was performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism Version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). A Student's *t*-test was used to analyze the differences between the two groups. Differences between multiple groups were analyzed by one-way analysis of variance (ANOVA), followed by a post-hoc Tukey test. $p < 0.05$ was considered a statistically significant difference.

Results

Cyclic Loading Can Induce Apoptosis in Chondrocytes

It has been suggested that mechanical stress mediates apoptosis in chondrocytes[5,11,20]. To further investigate this, a cyclic loading environment was generated *in vitro* to detect chondrocyte apoptosis under different degrees of mechanical stress. Compared with control group, mechanical stress significantly promoted ch drocyte apoptosis in an elongation-dep manner (Figure 1A). In addition, ch cyte exposure to 10% elongation and 1 lic loading for 12, 24, and 48 h result in inc cell apoptosis in a time-depend manner (ure 1B). Furthermore, chon ytes mulate with different degrees of ani stress for 24 h led to upregulated pro ession s cleaved-caspase-3 a eave as (Figure 1C), which h been repo critical markers in cell is[21]. These ngs indicated that m ss could induce chondrocyte apoptosis in and elongation-dependen ers.

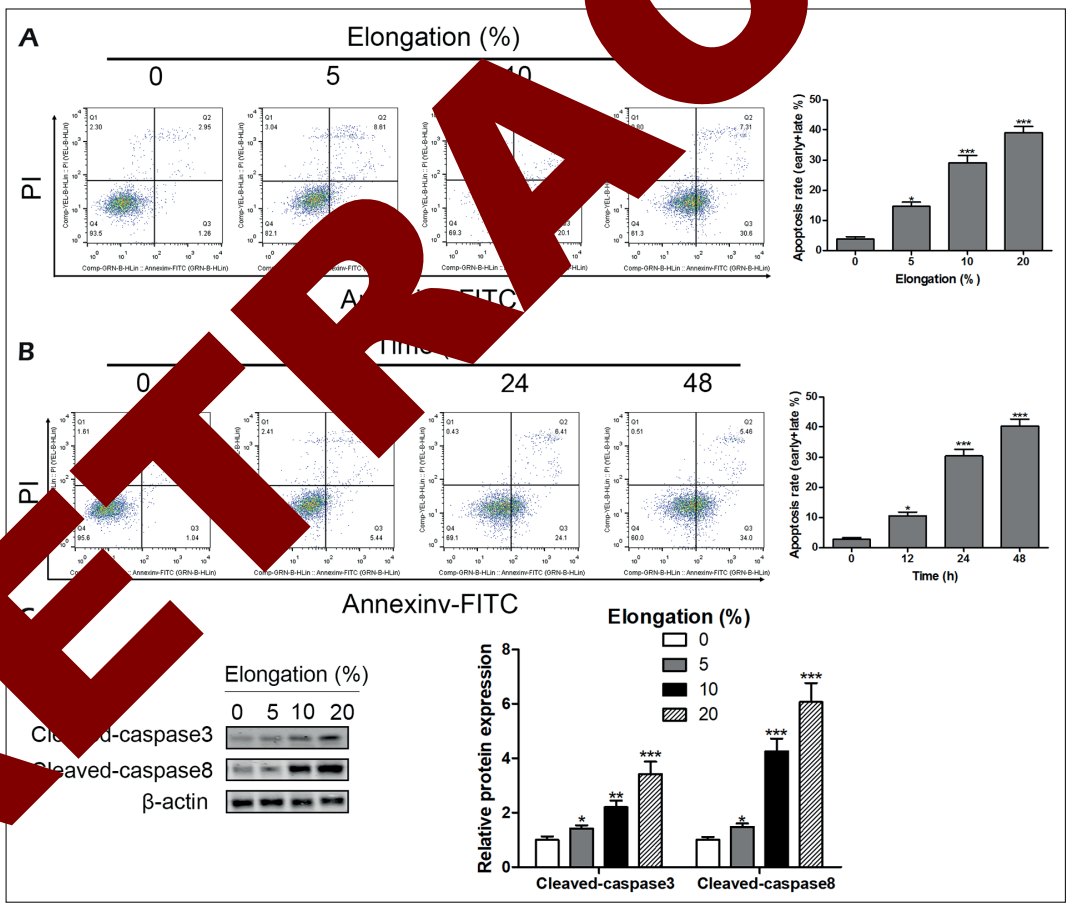

Figure 1. Cyclic loading induces the apoptosis of chondrocytes. Cell apoptosis was analyzed via flow cytometry using Annexin V-FITC/PI under different (**A**) elongation and (**B**) times. The protein expression of cleaved-caspase-3 and cleaved-caspase-8 was measured *via* Western blotting (**C**). *$p < 0.05$, ** $p < 0.01$, ***$p < 0.001$. n=3 in each group. FITC, fluorescein isothiocyanate; PI, propidium iodide.

Cyclic Loading Induces the Apoptosis of Chondrocytes by Upregulating GRP94

GRP94 is a marker of ERS, which has been reported to serve an important role in mechanical stress-induced cell dysfunction[11]. To further investigate whether GRP94 is associated with the mechanical stimulation-induced apoptosis of chondrocytes, ERS was triggered in chondrocytes via treatment with tunicamycin (Tun), as well as salubrinal (Sal), an inhibitor of Tun-induced ERS. The results indicated that Tun and cyclic loading could effectively induce the apoptosis of chondrocytes; treatment with Sal significantly reversed Tun or cyclic loading-induced apoptosis compared with Tun treatment or cyclic loading alone (Figure 2A and 2B). These findings suggest that mechanical stress-induced chondrocyte apoptosis may be associated with the activation of ERS. Additionally, the mRNA (Figure 2C) and [illegible] (Figure 2D) expression levels of GRP[illegible] were markedly upregulated by Tun and cyclic loading compared with control group; further treatment with Sal significantly reduced mRNA and protein expression levels of GRP94 compared with Tun treatment or cyclic loading alone (Figure 2C and 2D). These results suggest that GRP94, as a [illegible] is [illegible]tive

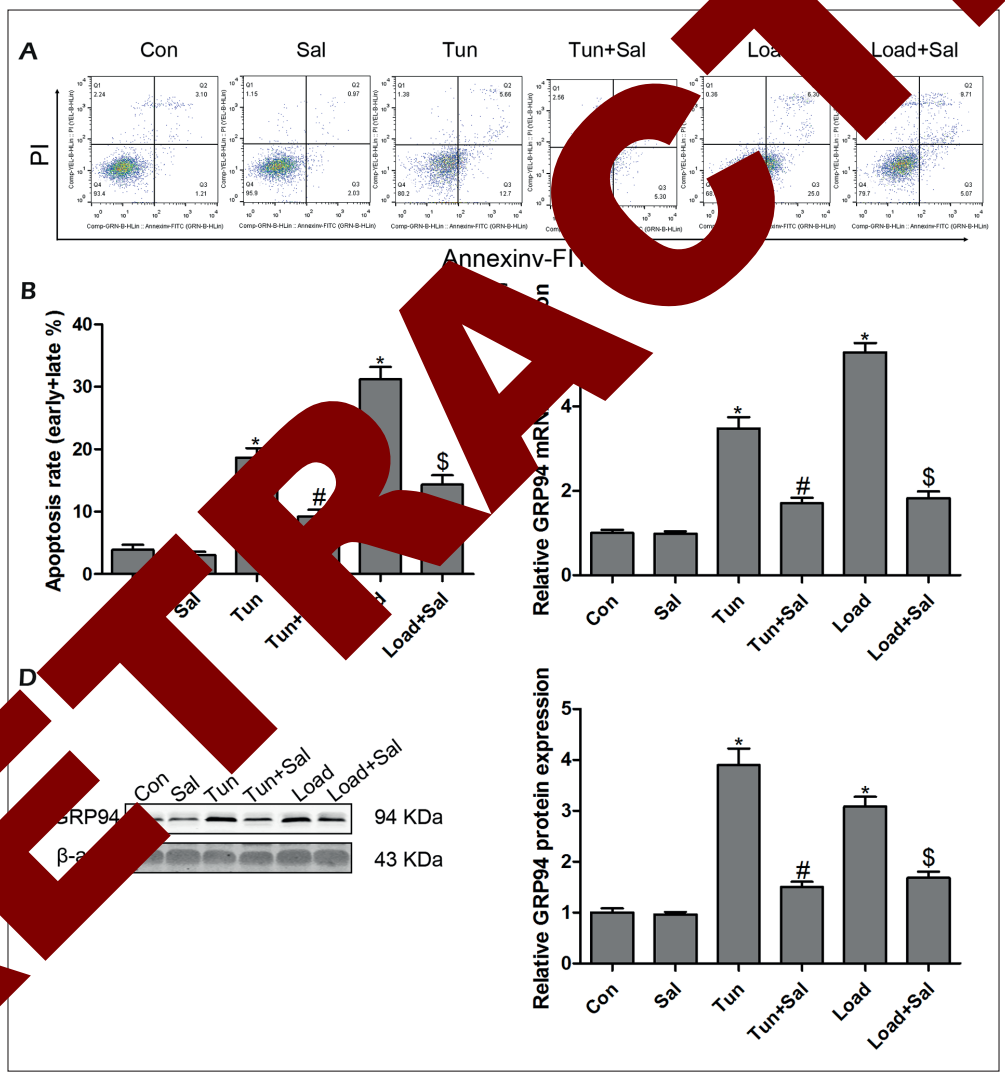

Figure 2. Cyclic loading induces the apoptosis of chondrocytes by activating ERS. **A,** and **B,** Following exposure to various conditions, chondrocyte apoptosis was analyzed by flow cytometry; the (**C**) mRNA and (**D**) protein expression levels of GRP94 were measured by reverse transcription-quantitative polymerase chain reaction and Western blotting, respectively. * p <0.05 *vs.* control group; # p <0.05 *vs.* Tun group; $ p <0.05 *vs.* load group. n=3 in each group. ERS, endoplasmic reticulum stress; GRP94, glucose-regulated protein 94; Tun, tunicamycin.

gene, may be regulated by cyclic loading and is involved in the mechanical stress-induced apoptosis of chondrocytes.

Prediction of Mechanical Stress-Regulated MiRNAs in Chondrocytes

To further investigate the post-transcriptional mechanism associated with mechanical stress-induced chondrocyte apoptosis, miRanda was used to identify potential miRNAs that could target to GRP94. A total of 16 candidate miRNAs were identified. Then, the expression levels of the 16 candidate miRNAs in chondrocytes were analyzed following cyclic loading. The results demonstrated that 6 miRNAs were upregulated and 9 miRNAs were downregulated in chondrocytes following cyclic loading. Based on the fold change in miRNA expression, miR-150 was selected for further investigation (Figure 3A). To determine whether expression of miR-150 was associated with mechanical stress-induced chondrocyte apoptosis, flow cytometry analysis with Annexin V-FITC/

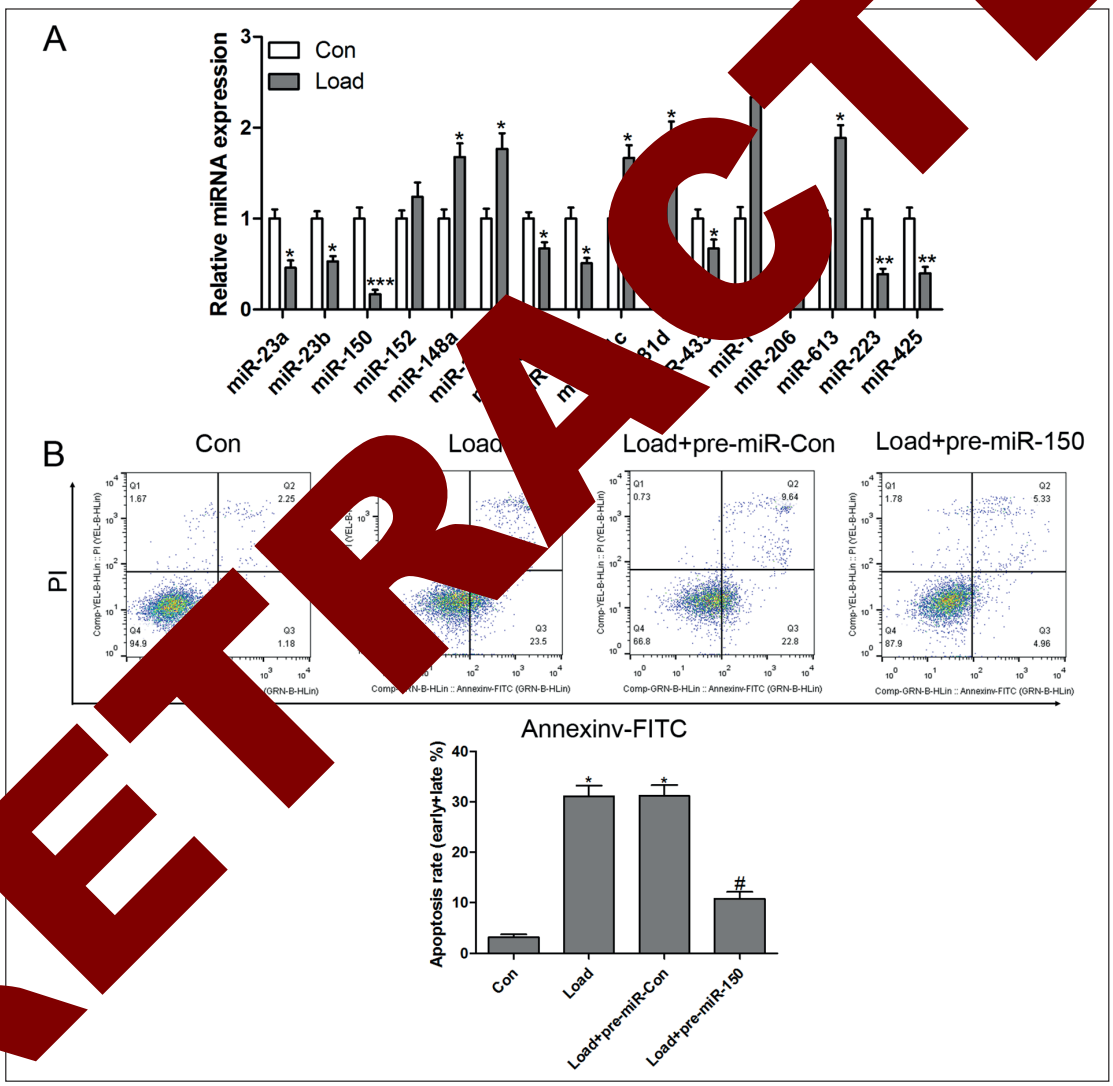

Figure 3. Overexpressed miR-150 suppresses cyclic loading-induced chondrocyte apoptosis. **A**, miRanda was used to identify potential miRs that target GRP94; the expression levels of 16 candidate miRs in mechanical stress-treated chondrocytes were measured by reverse transcription-quantitative polymerase chain reaction. **B**, Following transfection with pre-miR-150 in mechanical stress-treated chondrocytes, cell apoptosis was measured by flow cytometry. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ *vs.* control group; $^{\#}p<0.05$ *vs.* Load + pre-miR-Con group. n=3 in each group. Con, control; GRP94, glucose-regulated protein 94; miR, microRNA.

PI double staining of chondrocytes was performed. Cyclic loading-induced apoptosis was suppressed by overexpression of miR-150 in chondrocytes (Figure 3B).

GRP94 is a Direct Target Gene of MiR-150

Using miRanda, the 3'-UTR of GRP94 was determined to contain one conserved binding site of miR-150. The putative binding site for miR-150 in the 3'-UTR of GRP94 was presented in Figure 4A. To investigate whether miR-150 targeted the 3'-UTR of GRP94, the WT or MUT sequence of GRP94 inserted into a luciferase-reporter plasmid was co-transfected with pre-miR-Con, pre-miR-150, anti-miR-Con or anti-miR-150. Then, a luciferase reporter assay was performed. The results demonstrated that transfection with miR-150 mimics significantly decreased the luciferase activity (Figure 4B). MiR-150 inhibitors dramatically increased the luciferase activity in cells transfected plasmids containing the WT 3'-UTR of GRP94; these effects were not observed in cells transfected with the M 3'-UTR of GRP94 (Figures 4B and 4D addition, the mRNA and protein expres levels of GRP94 were significantly suppres in miR-150 mimics-transfected drocyt (Figure 4C). Conversely, the d pro tein expression levels of G 4 w signifi- cantly upregulated in ch yt transfection with mi 150 rs (e 4E). Collectively, t result cated that GRP94 is a dire et gene o 50.

Overexpression of Inhibits the P tive Effects iR-150 on hani l Stress-Induced C ro Apoptosis

f GR n miR-150-mediated protectio st anical stress-induced chondrocyte was investigated. MiR-150 mics an ector-GRP94 were co-transfected ndrocytes under the conditions of cyclic he results revealed that overexpressed GRP94 suppressed the protective effects of miR-50 mimics on mechanical stress-induced chondrocyte apoptosis (Figures 5A and 5B). These results indicated that miR-150 may serve a protective role in mechanical stress-induced chondrocyte apoptosis, at least partially, via the suppression of GRP94.

Discussion

Mechanical loading as an important pathogenic factor contributes to arthralgia, arthrocele, articular cartilage deterioration, and chon cyte death[11,22]; however, the molecular nisms underlying these pathological sses require further investigation. Emerging ce has suggested that certain miR , in miR-92, miR-153, miR-223 mi a, and i 568, are expressed in resp to hanic stress-induced dysfunctio vari of organs and cell types[23-25]. A comp anal of miRNA expression es h on d a significant increa the expre miR-9-5p, miR-138-5p 6a-5p, and 335-5p in the cartilage h OA[26]. The functional analysis of miRNAs l aled that miR-146a and mi are induced echanical loading, and eterious effects n articular cartilage an ondrocytes[5]. In the present study, it was re d that th pression of miR-150 was inhi y cycli ading in chondrocytes, while ove miR-150 protected against cyclic loa duced chondrocyte apoptosis *in* . Further investigation suggested that miR- post-transcriptional regulator, targeted pression of GRP94 suppressed by cyclic oading-induced ER dysfunction in chondrocytes.

MiR-150 has been frequently reported in various types of malignancy, and the majority of studies have indicated that miR-150 serves a beneficial role in inhibiting the growth of tumors[27,28]. In addition, miR-150 is a potential regulator in pathogenic infection and autoimmune diseases, including rheumatoid arthritis, ankylosing spondylitis, and systemic sclerosis[29,30]. MiR-150 is differentially expressed in chondrocytes during different stages of maturation, which suggests that miR-150 serves a crucial role in the development of cartilage and bone[31]. In the present study, the expression of miR-150 was downregulated in chondrocytes under conditions of mechanical stress and may serve a key role in chondrocyte apoptosis via the regulatory mechanism of negative feedback of GRP94 expression.

Additionally, miR-150 was proposed to directly target the 3'-UTR of GRP94 to inhibit mechanical stress-induced chondrocyte apoptosis, which is a classical post-transcriptional mechanism and is associated with ERS-mediated apoptosis in chondrocytes. ERS has been reported to serve an important role in mechanical stress-induced apoptosis[32]. In addition, mechanical stress induc-

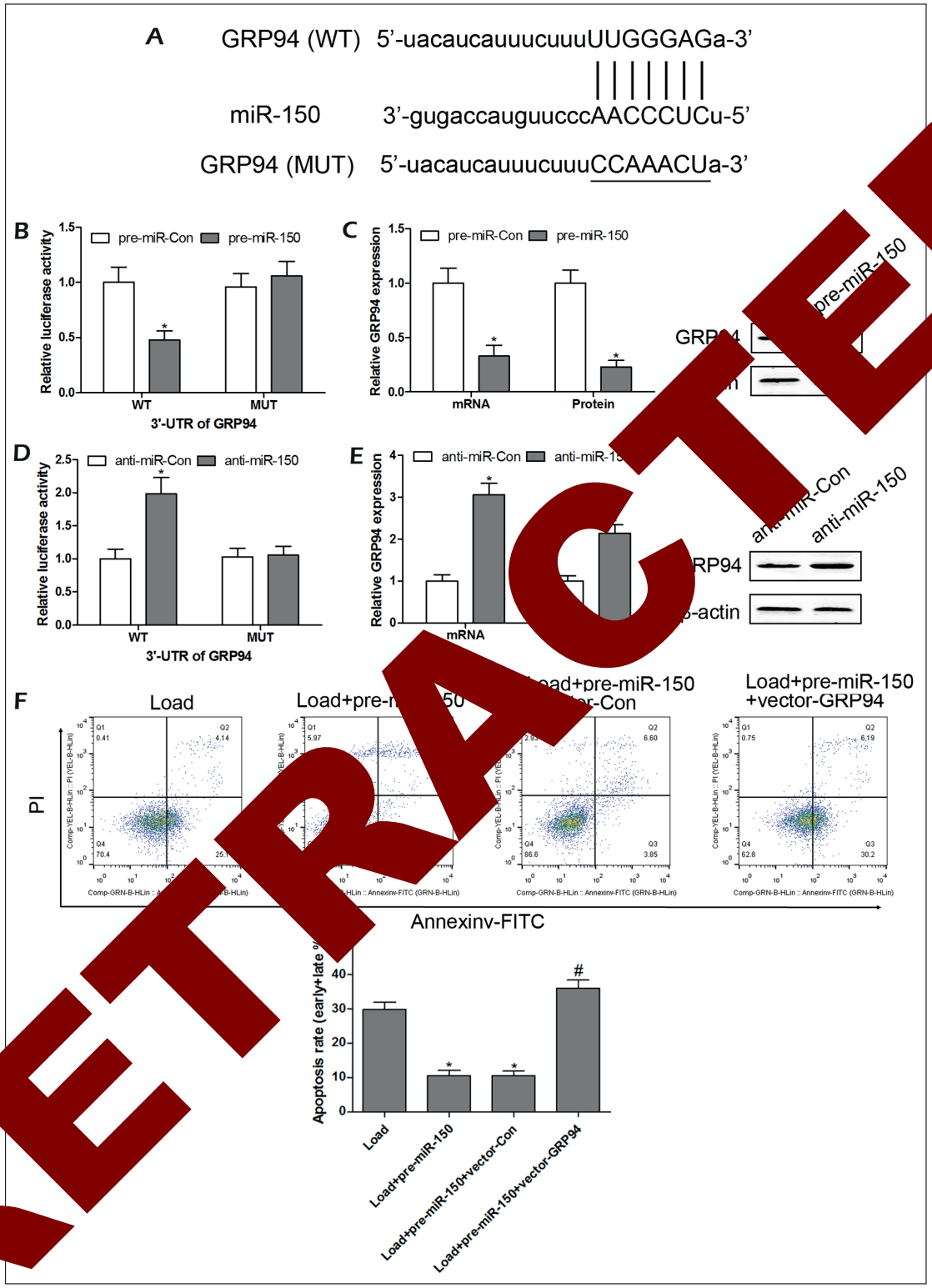

Figure 4. GRP94 is a direct target of miR-150. **A,** Putative miR-150 binding sites in the 3'-UTR of GRP94 were predicted using miRanda. **B, D,** Chondrocytes were co-transfected with plasmids containing the WT or MUT 3'-UTR of GRP94, and pre-miR-Con, pre-miR-150, anti-miR-Con or anti-miR-150; a luciferase activity assay was performed after 48 h post-transfection. **C, E,** Following transfection with pre-miR-Con, pre-miR-150, anti-miR-Con and anti-miR-150, the mRNA and protein expression levels of GRP94 in chondrocytes were measured by reverse transcription-quantitative polymerase chain reaction, and Western blotting, respectively; (**F**) cell apoptosis was measured by flow cytometry. * p <0.05. n=3 in each group.

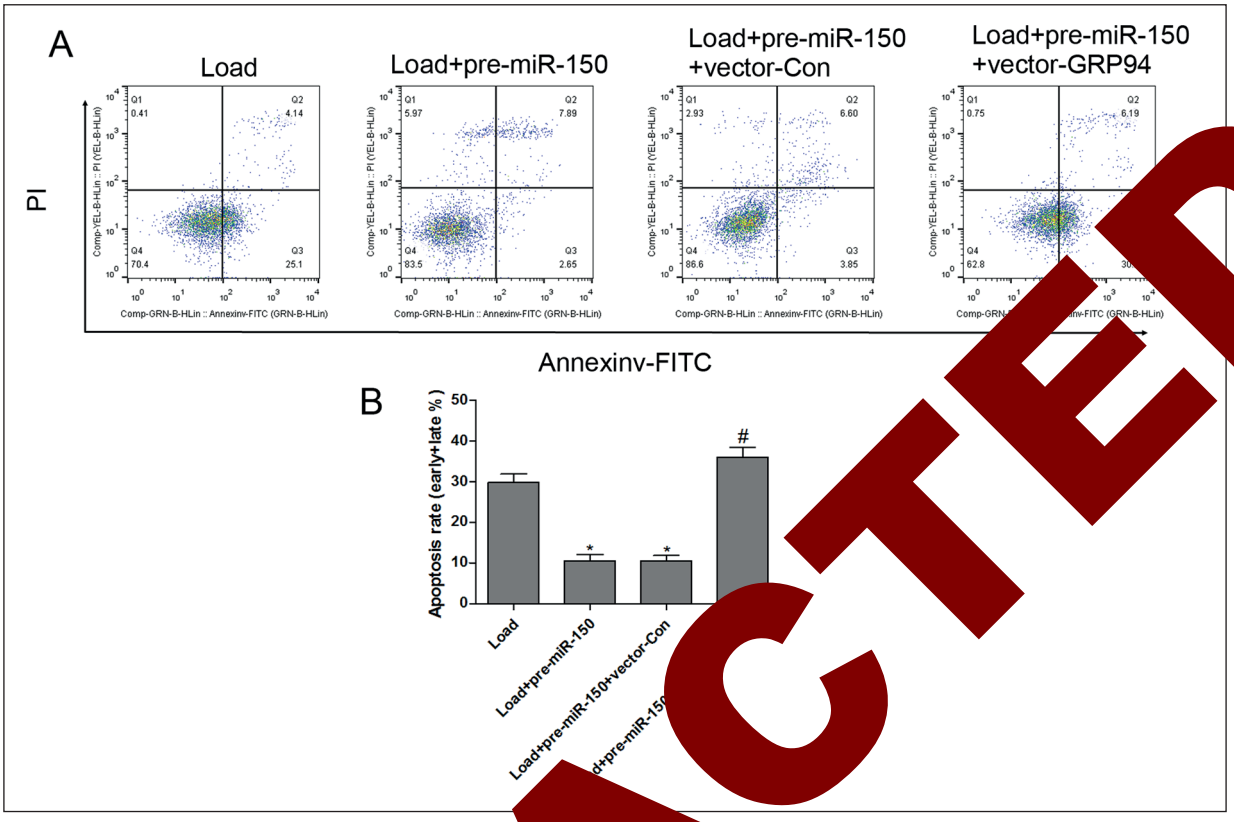

Figure 5. Overexpression of GRP94 neutralizes the [illegible]iR-150 in chondrocytes. Following transfection of pre-miR-150, or pre-miR-150 and vector-GRP94 in [illegible]eated chondrocytes, cell apoptosis was analyzed by flow cytometry (**A, B,**) * p <0.05 *vs.* control group; [illegible]. Load + pre-miR-150 + vector-Con group. n=3 in each group. Con, control; GRP94, glucose-re[illegible]rotein 9[illegible], microRNA; MUT, mutant; UTR, untranslated region; WT, wild-type.

es the apoptosis of [illegible]rocyt[illegible]he hyper-activation of ERS[illegible]uding the t[illegible]ation of GRP78, GRP94[illegible]ase 12 expre[illegible]ion, *in vitro* and *in vi*[illegible] hi[illegible]sistent with the findings of ou[illegible]esearch in v[illegible]echanical stress or ERS ac[illegible]ion increased [illegible]RNA and protein expr[illegible]n of [illegible]P94 in chondrocytes, while ERS a[illegible]ism[illegible]uced the expression of GRP94 inc[illegible]mecha[illegible] stress or ERS activation. Furth[illegible] th[illegible]uction of apoptosis was ac-mpani[illegible]e upregulation of GRP94, sug-ting tha[illegible]chanical stress-induced apoptosis [illegible]modulated by the hyperactivation of ERS. [illegible]ly, our data revealed that miR-150 may serve as an endogenous inhibitor to suppress e expression of GRP94 and protect chondro-tes from mechanical stress-induced apoptosis. These results also suggested that miR-150 may be inhibited by mechanical stress-enhanced ERS. Heindryckx et al[33] indicated that ERS-induced fibrotic diseases via the upregulation of RNase activity suppresses miR-150 expression, which shows that increased levels of miR-150 may serve a protective role in the dysfunction of ERS-induced organs or cells.

The present study demonstrated a critical role of miR-150 in mechanical stress-induced chondrocyte apoptosis, which may be due to the regulation of GRP94 expression; miRNAs have been reported to regulate numerous mRNAs in a variety of pathologic conditions. For example, miR-150 inhibits the progression of neuropathic pain by targeting zinc finger E-box binding homeobox 1[34]. MiR-150 as a post-transcriptional regulator of perforin-1, regulates the cytotoxicity of natural killer cells[35]. To the best of our knowledge, the present investigation is the first to report GRP94 as a direct target of miR-150 in mechanical stress-induced chondrocyte apoptosis. Other genes may be regulated by miR-150 in this process; however, further research is required.

Conclusions

Collectively, these findings suggested that miR-150, as a post-transcriptional regulator of GRP94, inhibits the mechanical stress-induced apoptosis of chondrocytes. Thus, miR-150 and GRP94 may serve as potential therapeutic targets for the treatment of OA.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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Authors' Contribution

ZZ and KW made substantial contributions to the design of the present the study. ZZ, CW and PY conducted literature searching, and data acquisition and analysis. ZZ, CW, PY and KW performed the in vitro experiments, and prepa edited and reviewed the manuscript. All authors re approved the final version of the manuscript.

Ethics Approval and Consent t ipate

The present study was approved by mmittee of The Second Affiliated Hospital an Jia g Univer-sity (Xi'an, China). Written info nse from all of participants prior to sa

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