Silencing of long-chain non-coding RNA GAS5 in osteoarthritic chondrocytes is mediated by targeting the miR-34a/Bcl-2 axis

QINGHUI JI[1,2], XIAOFENG QIAO[2], YONGXIANG LIU[3], DAWEI WANG[4] and JINGLONG YAN[1]

[1]Department of Orthopedics, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150086; [2]Department of Orthopedics, The First Affiliated Hospital of Jiamusi University, Jiamusi, Heilongjiang 154000; [3]Department of Orthopedics, Hegang People's Hospital, Hegang, Heilongjiang 154100; [4]Department of Orthopedics, First Hospital of Zhangjiakou, Zhangjiakou, Hebei 075000, P.R. China

Received July 11, 2019; Accepted November 18, 2019

DOI: 10.3892/mmr.2019.10900

Abstract. The present study aimed to investigate the effects of the long non-coding RNA (lncRNA) growth arrest-specific transcript 5 (GAS5) on proliferation, apoptosis and the inflammatory response of osteoarthritic chondrocytes (OACs) and its associated mechanism of action. Primary chondrocytes were isolated from cartilage tissues of osteoarthritis (OA) patients for subculture. GAS5 was silenced in OACs by liposome transfection. The effects of GAS5 silencing on proliferation, apoptosis, stromal metabolism and inflammatory response of OACs were analyzed. The association of GAS5 with its target microRNA-34a (miR-34a) and the downstream target gene *Bcl-2* was verified by luciferase reporter assays. The results indicated that GAS5 silencing promoted the proliferation, inhibited cell apoptosis and caused G1 arrest of OACs compared with the control group ($P<0.05$). The expression levels of TNF-α and IL-6 in the supernatant of OACs in the si-GAS5 group were significantly lower than those of the control group ($P<0.05$). The results of the double luciferase reporter assays indicated that overexpression of GAS5 downregulated miR-34a and upregulated Bcl-2 levels ($P<0.05$) compared with the expression levels of these markers in the control group. In contrast to GAS5 overexpression, knockdown of this RNA caused a significant upregulation of miR-34a levels and a significant downregulation in the levels of Bcl-2 ($P<0.05$). Moreover, GAS5 overexpression could counteract the inhibition of apoptosis by overexpression of miR-34a ($P<0.05$). The data indicated that GAS5 participated in the development of OA by regulating the biological behavior of chondrocytes via the miR-34a/Bcl-2 axis.

Correspondence to: Dr Jinglong Yan, Department of Orthopedics, The Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Nangang, Harbin, Heilongjiang 150086, P.R. China
E-mail: yanjinglong_gk@outlook.com

Key words: lncRNA GAS5, osteoarthritis, chondrocytes, mechanism, miR-34a/Bcl-2

Introduction

Osteoarthritis (OA) is a degenerative disease characterized by cartilage degeneration, osteophyte formation, and narrowing of the joint space. This disease has become the fourth most disabling cause in the world (1). A major focus in bone research has been the understanding of the etiology and pathogenesis of OA. Recent studies have revealed that long non-coding RNAs (lncRNAs) are associated with a variety of diseases, including OA (2-4). The lncRNA growth arrest-specific transcript 5 (GAS5) is one of the most important lncRNAs noted in human T lymphocytes and in non-transformed lymphocytes, and its current functional annotation is that of a tumor suppressor gene currently (5). LncRNAs can exert their biological functions by targeting miRNAs. Previous studies that investigated GAS5 and its target miRNAs have mainly focused on cancer (6,7). A limited number of studies have explored the role of GAS5 in the development of OA (8). In the present study, GAS5 expression was investigated with regard to chondrocyte proliferation, apoptosis, extracellular matrix (ECM) metabolism and inflammatory response by silencing its expression in osteoarthritic chondrocytes (OACs).

Materials and methods

Source of specimens. A total of 30 patients who underwent knee arthroplasty from January 2016 to June 2018 at the Second Affiliated Hospital of Harbin Medical University were enrolled (OA group). In addition, 30 patients with artificial hip arthroplasty due to femoral neck fracture were selected as the control group. Cartilage tissue samples of OA and control patients were obtained during surgery. The cartilage tissue was stored in a sample bottle of phosphate-buffered saline (PBS) solution containing 20% medium and 5% calf serum for chondrocyte culture. The present study was approved by the Second Affiliated Hospital of Harbin Medical University Ethics Committee and all subjects signed the relevant informed consent.

Isolation and culture of chondrocytes. The cartilage tissue was cut into small pieces of approximately 1 mm^3 in diameter using a pair of ophthalmic scissors. The tissue fragments

were digested and centrifuged (250 x g for 15 min at room temperature), the supernatant was discarded and the primary chondrocytes were isolated from the precipitate. The collected primary chondrocytes were resuspended in RPMI-1640 complete medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin. The cells were cultured at 37˚C, in the presence of 5% CO_2. The culture medium was replaced every two days. The chondrocytes were identified by toluidine blue staining and immunocytochemical analysis. The subculture was carried out when the cells were grown to 80% confluence. The samples from the second-generation chondrocytes were obtained for subsequent experiments.

Toluidine blue staining. Following routine digestion, the chondrocytes were inoculated in 24-well culture plates and pre-plated with glass slides. The chondrocytes were collected and the culture solution was discarded. The cells were placed in 4% paraformaldehyde and fixed at 4˚C for 1 h. The slides were washed with tap water for 15 min and placed in toluidine blue dye solution for 2 h. Excess dye solution was removed and the slides were placed under an inverted microscope for visualization.

Transfection. The second generation OACs were obtained for further experiments. The following groups were included: Control, si-NC and si-GAS5. The cells were transfected with GAS5 siRNA and the corresponding GAS5 siRNA negative sequences using Lipofectamine 2000 transfection reagent. The molecular mechanism of GAS5 that affected chondrocyte apoptosis in OA was examined. miR-34a overexpression or inhibitory cell lines (miR-34a mimic and its negative control mimic-NC, as well as miR-34a inhibitor and its negative control inhibitor-NC) were used for further experiments. In addition, a GAS5 overexpression or knockout cell line (transfection of OA articular chondrocytes with GAS5 and sh-GAS5 plasmids to overexpress or knockdown intracellular GAS5) and a co-transfected cell model that overexpressed GAS5 and miR-34a (miR-34a mimic and its negative control mimic-NC, as well as GAS5 and its negative control sh-GAS5) were established. All protocols were carried out in accordance with the manufacturer's instructions. Following transfection and incubation for 24 h, the transfection efficiency was verified by reverse transcription-quantitative PCR (RT-qPCR) detection of GAS5.

RT-qPCR. Total RNA was extracted following cell transfection. RNA extraction was conducted using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) in an RNAase-free environment. The total RNA concentration and purity were detected by the ultra-micro nuclear protein assay. A quantitative PCR amplification instrument was used to detect the expression levels of the objective gene. The primers sequences of GAS5,miR-34a and Bcl-2 were designed and synthesized by Shanghai Sangon Biotech (Table I). The reaction conditions were as follows: preheating at 50˚C for 2 min and initial denaturation at 95˚C for 10 min. A total of 35 cycles were performed that included the following steps: Denaturation at 95˚C for 10 sec, annealing at 60˚C for 1 min and extension at 72˚C for 55 sec. The analysis was carried out by the $2^{-\Delta\Delta Cq}$ (9) method.

MTT assay for the detection of chondrocyte growth. The cells were cultured for 48 h following transfection. The cell density was adjusted at $2x10^4$ cells/ml and the cells were transferred into 96-well plates. A total of 5 replicate wells were set in each group and cultured at 37˚C in the presence of 5% CO_2. A total of 20 μl MTT solution (5 mg/ml) was added to each well. Following continuous culture for 4 h, 150 μl of dimethyl sulfoxide (DMSO) was added to each well, shaken and mixed for 10 min at room temperature. The absorbance value (OD) of each group of cells was measured at 490 nm. The measurements were repeated three times per well.

Flow cytometry (FCM) for apoptosis detection and cell cycle analysis. The cells were cultured for 48 h following transfection. The samples were centrifuged at 1,000 x g for 5 min at room temperature and 500 μl of buffer was added to adjust the cell suspension concentration to $1x10^6$ cells/ml. A total of 5 μl of Annexin V-FITC was added and mixed with the samples. The samples were incubated at 4˚C for 15 min in the dark. A total of 5 μl of PI staining solution was added and the samples were incubated at 4˚C for 5 min in the dark. Apoptosis was detected by FCM. The cell suspension was transferred to a 1.5-ml centrifuge tube, and 95 μl pre-cooled 75% ethanol was added. The final samples were incubated at 4˚C for 24 h. Following centrifugation at 1,000 x g for 5 min at room temperature, the supernatant was discarded and the cells were resuspended in PBS. A total of 500 μl PI staining solution (0.5%) was added and the cells were resuspended and incubated in the dark. The cell cycle was detected by FCM.

Western blot analysis. Following transfection, each group of cells was collected. The cells were lysed with RIPA cell lysate (Beyotime Institute of Biotechnology) and centrifuged at 12,000 x g for 10 min at 4˚C. The protein concentration was determined by BCA. Protein (40 μg) was separated by 10% SDS-PAGE and transferred into polyvinylidene difluoride (PVDF) membranes. The membranes were blocked by TBST (0.25%Tween) with 5% skimmed milk for 2 h at room temperature, and incubated with the corresponding primary antibodies (Invitrogen; Thermo Fisher Scientific, Inc.): Anti-Bcl2 (1:1,000, cat. no. 138800), Aanti-Bax (1:1,000, cat. no. BMS163), anti-MMP3 (1:5,000, cat. no. MA514247), anti-Collagen II (1:5,000, cat. no. MA512789), anti-Aggrecan (1:1,000, cat. no. MA316888), anti-Ki-67 (1:200, MA514520), anti-PCNA (1:100, MA511358), anti-CDK2 (1:2,000, cat. no. MA532017), Anti-p53 (1:1,000, cat. no. MA512557), at 4˚C overnight. The secondary antibodies Invitrogen; Thermo Fisher Scientific, Inc.): Goat anti-mouse HRP-IgG (1:10,000, cat. no. G21040) or Goat anti-rabbit HRP-IgG (1:10,000, cat. no. G21234) were incubated for 2 h at room temperature. The ECL luminescent agent (Tiangen Biotech Co., Ltd.) was added for film development in a dark room. β-actin was used as an internal reference. The analysis was performed by Quantity-One software (v4.6.6; Bio-Rad Laboratories, Inc.).

Enzyme-linked immunosorbent assay (ELISA). The expression levels of the inflammatory factors IL-6 and TNF-α were assessed by ELISA in chondrocytes derived from osteoarthritis patients. The experiments were performed step by step in strict accordance with the kit instructions.

Table I. Primer sequences.

Primer name	Sequences
GAS5	(F) 5'-TCGGCTTGACTACACTGTGT-3'
	(R) 5'-GGAGGCTGAGGATCACTTGA-3'
Bcl-2	(F) 5'-GAGCATCTCACACTCGTTG-3'
	(R) 5'-GAAAGAGGGATGCTGTCTCG-3'
β-actin	(F) 5'-CATGGAATCTGTGGCATGG-3'
	(R) 5'-TGATCTTCATGGTGCTGGGA-3'
miR-34a	Stem loop: 5'-AGCTCAGAAGCTGCCACAAT-3'
	(F) 5'-TTCAAGAACACCTGCACAGC
	(R) 5'-GGAAAGTACGCAGCCAAGTC

F, forward; R, reverse.

Luciferase reporter assay. The binding site of GAS5 on miR-34a was predicted by the Starbase v2 software (http://starbase.sysu.edu.cn/index.php), and the binding site of miR-34a and Bcl-2 was predicted by TargetScan (http://www.targetscan.org/vert_72/). PCR amplified a fragment of the miR-34a binding site in GAS5 and Bcl-2, respectively. The corresponding fragments were inserted into the pcDNA-Report vector, respectively. The cells were co-transfected with the miR-34a mimic or the mimic-NC using GAS5-MUT (or GAS5-WT) and Bcl-2-MUT (or Bcl-2-WT) plasmids, respectively. Fluorescence intensity was determined according to the Dual Luciferase reporter kit (Invitrogen; Thermo Fisher Scientific, Inc.). All experiments were carried out in strict accordance with the manufacturer's instructions.

Statistical analysis. All data in this study were processed using SPSS 20.0 statistical software (IBM Corp.). All data are expressed as the mean ± standard deviation (SD). Comparison among multi-groups were conducted by ANOVA and pairwise comparisons were performed using the LSD t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Primary culture and identification of chondrocytes. The morphology of articular chondrocytes in the two groups and in the second-generation chondrocytes were stained with toluidine blue and observed using microscopy. The chondrocytes in the two groups were normal in morphology and exhibited a long fusiform structure (Fig. 1A). Following staining with toluidine blue, the nuclei of the cells were stained dark blue and the nucleolus was visible (Fig. 1B). The data indicated that the chondrocytes in the normal group exhibited higher density than those of the OA group.

Comparison of lncRNA GAS5 expression in normal chondrocytes and OACs. The expression of GAS5 in primary cultured chondrocytes was detected by RT-qPCR. GAS5 was expressed in both articular and normal chondrocytes and its expression levels in the osteoarthritic chondrocytes was significantly higher than those noted in normal chondrocytes (2.37±1.12 vs. 1.07±0.22) ($P<0.05$, data not shown).

Transfection efficacy. The transfection efficacy was verified by RT-qPCR. The expression levels of GAS5 in the si-GAS5 group were significantly lower compared with those of the control group ($P<0.05$). The expression levels in the si-NC and control groups exhibited no significant difference ($P>0.05$, Fig. 2A).

The expression levels of miR-34a were significantly higher in the miR-34a mimic group, while the miR-34a inhibitor group exhibited significantly lower miR-34a levels than those of the control group (Fig. 2B). No significant differences were noted in the expression levels of miR-34a between the mimic-NC, the inhibitor-NC and the control groups ($P>0.05$). GAS5 overexpression or knockout in transfected cell lines (GAS5 or sh-GAS5) was successfully established in chondrocytes (Fig. 2C).

Effects of GAS5 silencing on chondrocyte proliferation, cell cycle and apoptosis induction. The damage caused in articular cartilage is considered the pathological basis of OA. During that process, chondrocyte proliferation and apoptosis play an important role (10). MTT assay demonstrated that the proliferation of cells in the si-GAS5 group was significantly increased compared with that of the control group ($P<0.05$). Western blot analysis indicated that the expression levels of proliferating cell nuclear antigen (PCNA) and Ki-67 in the si-GAS5 group were significantly higher than those of the control group ($P<0.05$, Fig. 3A). The results suggested that GAS5 silencing could promote the proliferation of OACs.

FCM analysis indicated that the apoptosis rate of OACs in the si-GAS5 group was significantly lower than that of the control group following transfection and cell culture for 48 h (Fig. 3B, $P<0.05$). The expression levels of Bax in the si-GAS5 group were significantly increased, while the expression levels of Bcl-2 were significantly decreased ($P<0.05$, Fig. 3B). The results of the FCM analysis further demonstrated that the percentage of G0/G1 phase cells in the si-GAS5 group was increased, while the number of cells in the G2/M phase decreased significantly compared with that of the control group (Fig. 3C, $P<0.05$). Western blot analysis indicated that the expression levels of CDK2 in the si-GAS5 group was significantly lower than that in the control group, while the expression of p53 was higher than that in the control group (Fig. 3C, $P<0.05$). These results indicated that silencing of GAS5 could cause G1 arrest, promote proliferation of OACs and inhibit the induction of apoptosis.

Effects of GAS5 silencing on ECM metabolism and inflammatory response in OACs. Articular cartilage ECM protects chondrocytes from mechanical stress. The most important component is collagen II, followed by proteoglycans. Excessive degradation and loss of ECM is one of the important signs of the onset of OA (11). The results indicated that the expression levels of type II collagen and aggrecan were significantly increased in the si-GAS5 group compared with those of the control group, while the expression levels of MMP-13 were significantly decreased ($P<0.05$, Fig. 4A), indicating that GAS5 silencing inhibited the degradation of the cartilage matrix.

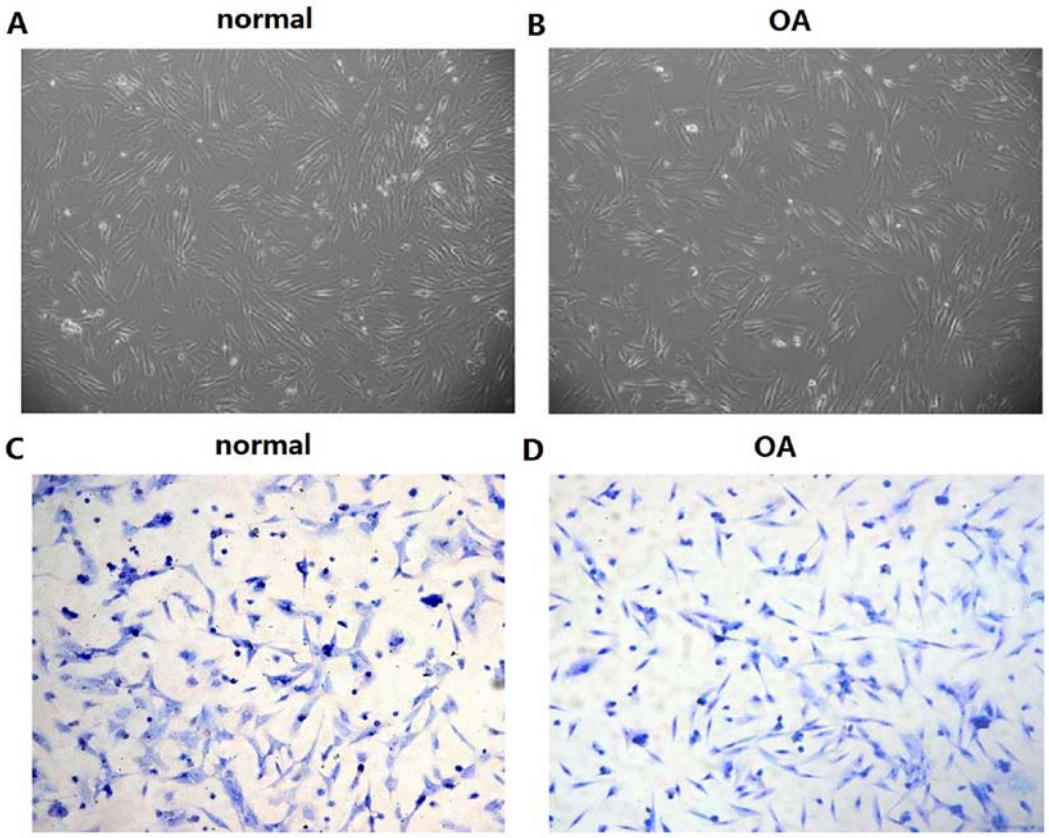

Figure 1. Primary culture and identification of chondrocytes. (A) Primary cultured normal chondrocytes. (B) Primary cultured OACs. (C) Toluidine blue staining of normal chondrocytes. (D) Toluidine blue staining of OACs. Magnification, x200. OACs, osteoarthritic chondrocytes.

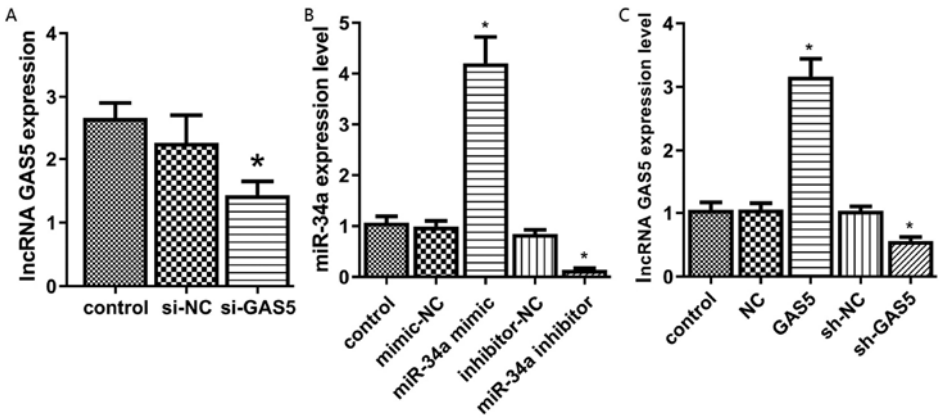

Figure 2. Identification of transfection effects by RT-qPCR. (A) Identification of the transfection effect of si-GAS5. (B) Identification of miR-34a overexpression and suppression cell models. (C) Identification of GAS5 overexpression or knockout cell models. *P<0.05 vs. control. RT-qPCR, reverse transcription-quantitative PCR; GAS5, growth arrest-specific transcript.

The inflammatory response plays an important role in the development of OA (12). ELISA indicated that the levels of TNF-α and IL-6 in the si-GAS5 group were significantly lower than those of the control group (P<0.05, Fig. 4B), indicating that GAS5 silencing could reduce the severity of the inflammatory response of OACs.

GAS5 targets miR-34a and regulates the expression of Bcl-2. It was predicted by TargetScan that miR-34a may be a target of GAS5 and Bcl-2 a candidate target gene of miR-34a (Fig. 5A). The results of the dual luciferase reporter assay demonstrated that luciferase activity was significantly decreased in cells co-transfected with GAS5-WT and miR-34a mimic (P<0.05, Fig. 5B). Bcl-2-WT and miR-34a mimic co-transfection resulted in a significant decrease in luciferase activity (P<0.05). GAS5 overexpression or knockout cell lines were established. Moreover, miR-34a overexpression was achieved by transfection of miR-34a to the cells, and miR-34a inhibition by addition of a miR-34a inhibitor. The results indicated that miR-34a was downregulated in the GAS5 group (Fig. 5C), while the expression levels of the Bcl-2 protein were increased compared with those of the control group (P<0.05, Fig. 5C).

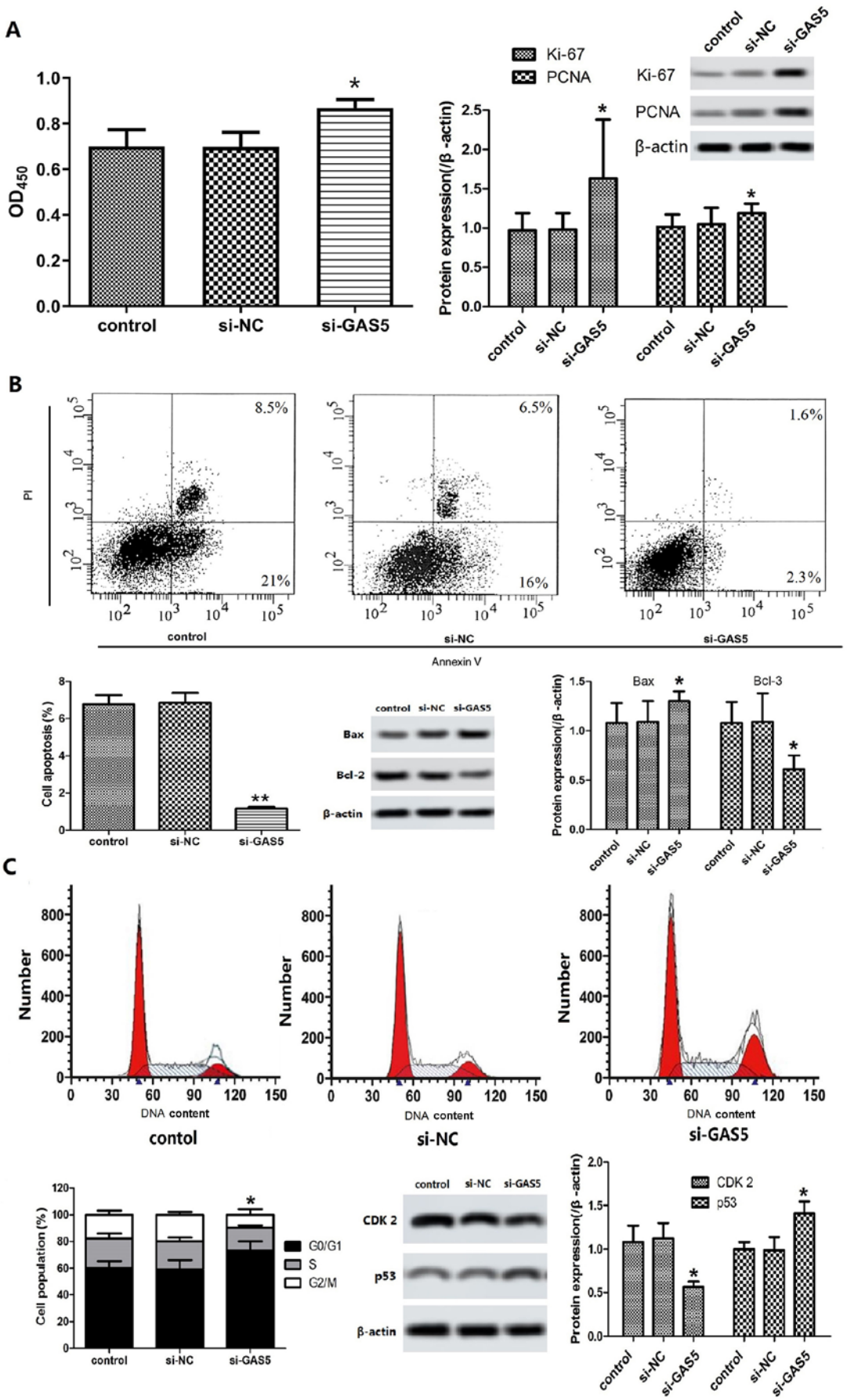

Figure 3. Effect of GAS5 silencing on proliferation, apoptosis and the cell cycle of OACs. (A) Cell proliferation and the protein expression levels of PCNA and Ki-67 by western blotting. (B) Cell apoptosis rate by FCM and the protein levels of Bax and Bcl-2 by western blotting. (C) Cell periodic distribution by FCM and the protein expression levels of CDK2 and p53 by western blotting. *P<0.05; **P<0.01 vs. control. GAS5, growth arrest-specific transcript; OACs, osteoarthritic chondrocytes; PCNA, proliferating cell nuclear antigen; FCM, flow cytometry.

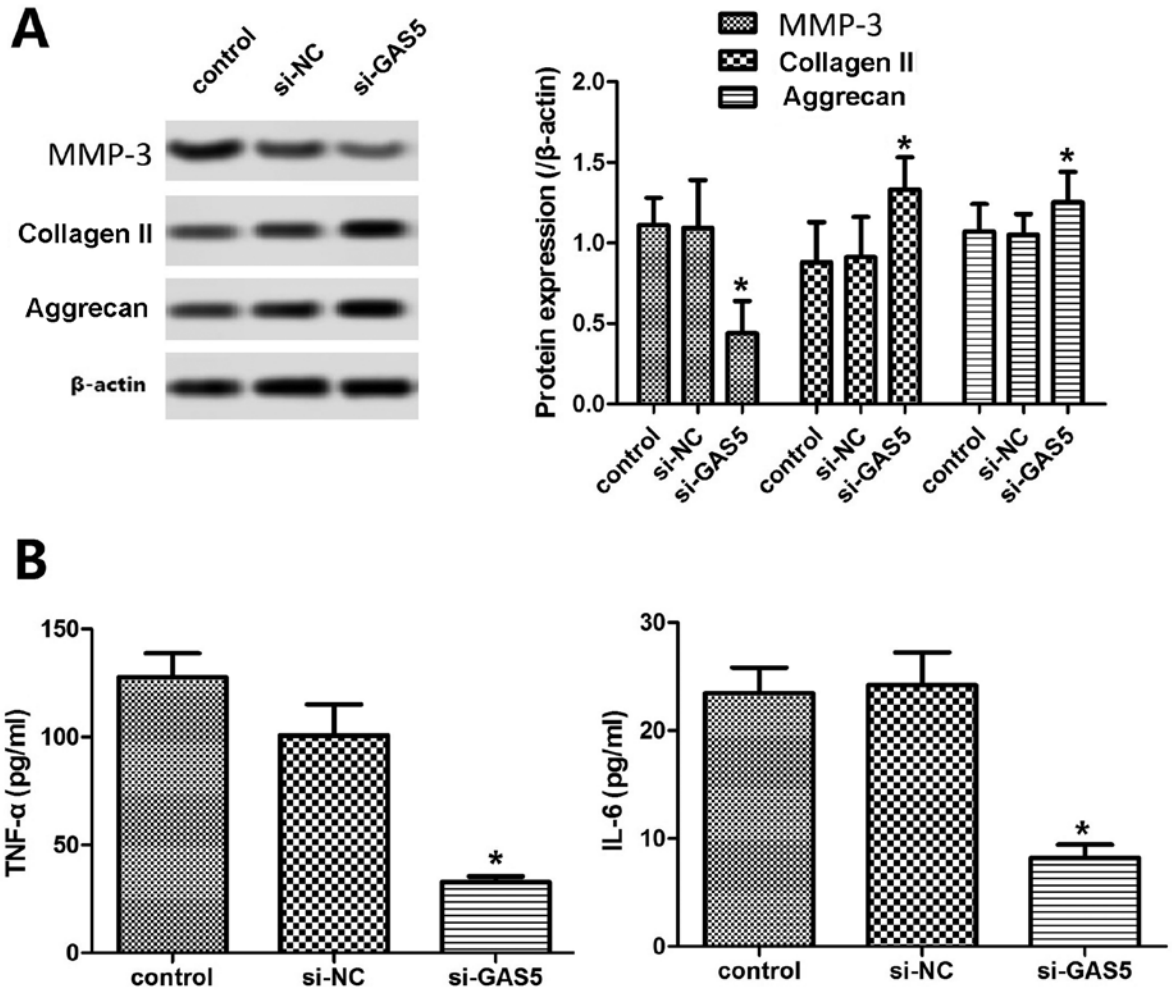

Figure 4. Effect of GAS5 silencing on ECM metabolism and inflammatory response in OACs. (A) Expression of MMP-13, collagen II and aggrecan by western blotting. (B) The content of TNF-α and IL-6 in the cell supernatant was analyzed by ELISA. *P<0.05 vs. control. GAS5, growth arrest-specific transcript; ECM, extracellular matrix; OACs, osteoarthritic chondrocytes; MMP, matrix metalloproteinase.

The effect noted in the sh-GAS5 group was contradictory to these findings. The expression levels of Bcl-2 in the miR-34a mimic and miR-34a inhibitor groups were lower and higher than those of the control group, respectively (P<0.05, Fig. 5D). These results indicated that GAS5 could indirectly regulate the expression levels of Bcl-2 by targeting miR-34a.

GAS5 targets miR-34a/Bcl-2 to regulate apoptosis. Cell lines that were co-transfected with GAS5 and miR-34a were established. The results of the FCM analysis indicated that the apoptotic rate of the GAS5 group was significantly increased compared with that of the control group (P<0.05, Fig. 6A), suggesting that GAS5 overexpression promoted the induction of apoptosis. The apoptotic rate in the miR-34a mimic with GAS5 overexpression group was lower than that of the GAS5 group alone and higher than that of the miR-34a mimic group alone (P<0.05). Consequently, the expression levels of Bcl-2 were significantly increased and significantly decreased in the GAS5 and in the miR-34a mimic groups, respectively (P<0.05, Fig. 6B). These findings indicated that the induction of OAC apoptosis by GAS5 was reduced by miR-34a and that the Bcl-2-mediated inhibition of miR-34a expression was reduced by GAS5. Collectively, the data indicated that GAS5 regulated the induction of OAC apoptosis by targeting the miR-34a/Bcl-2 axis.

Discussion

The main process involved in the pathogenesis of OA is the degeneration of articular cartilage, in which chondrocyte proliferation and apoptosis play an important role (13,14). Under normal circumstances, the processes of proliferation and apoptosis of chondrocytes are coordinated in an orderly manner in cartilage tissues. During excessive induction of chondrocyte apoptosis, internal cartilage disorders may occur, leading to abnormal cartilage function (15). Blanco *et al* (16) demonstrated that the proportion of apoptotic chondrocytes in the osteoarthritic cartilage was significantly higher than that noted in normal tissues (11% vs. 5.1%, P<0.01), which confirmed that chondrocyte apoptosis was associated with OA. Several lncRNAs have been revealed to be closely associated with the proliferation and apoptosis of chondrocytes. Li *et al* (17) revealed that PVT1 expression in OACs was significantly increased, while inhibition of PVIT1 could inhibit cell apoptosis. Zhang *et al* (18) indicated that the expression of UFC in OACs was downregulated and that this

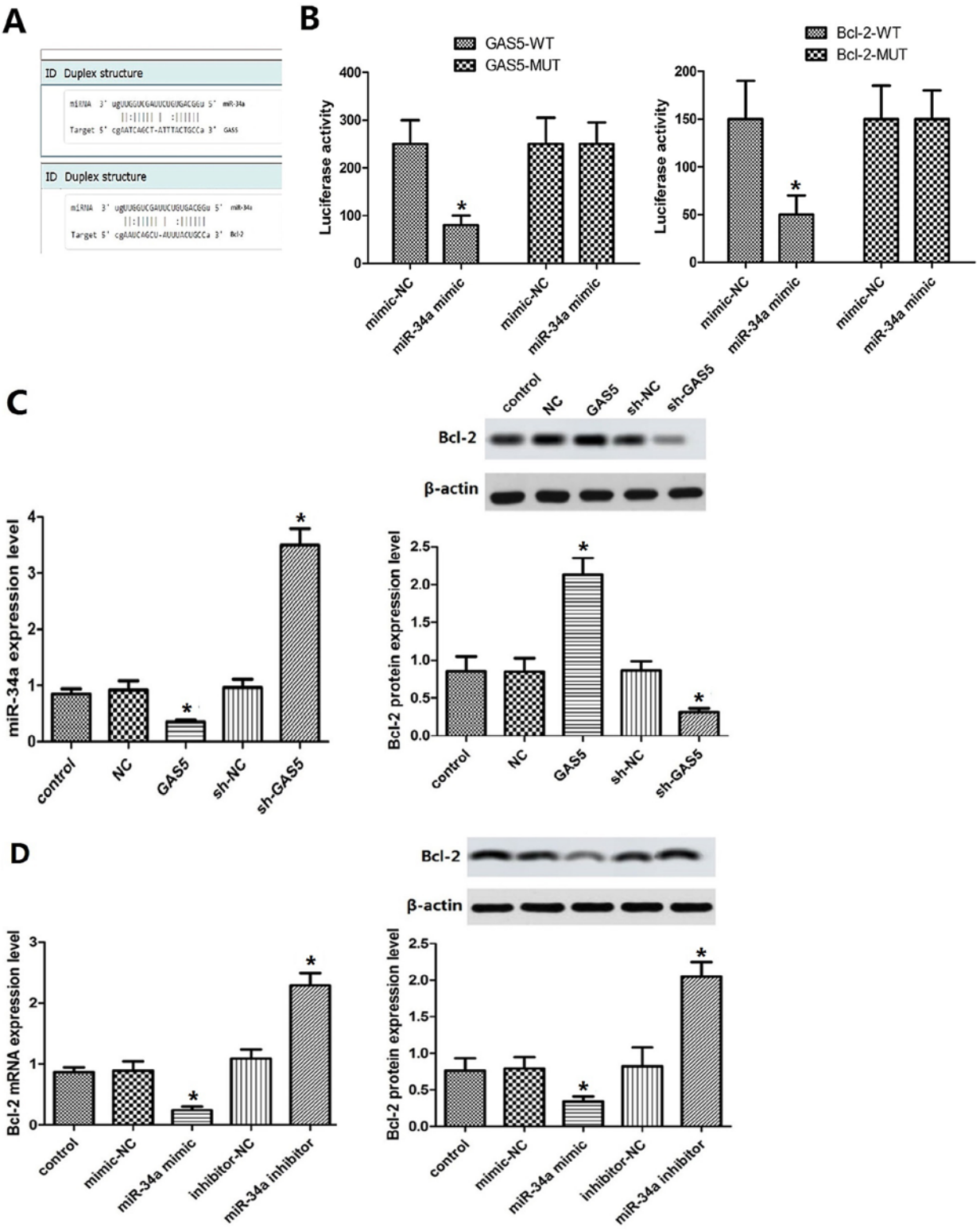

Figure 5. GAS5 targeting binding to miR-34a regulates Bcl-2 expression. (A) Bioinformatics analysis results of target genes. (B) Interaction between GAS5 and miR-34a, miR-34a and Bcl-2. (C) Effect of GAS5 on miR-34a and Bcl-2 expression. (D) Effect of miR-34a on Bcl-2 mRNA and protein expression. *P<0.05 vs. control. GAS5, growth arrest-specific transcript.

process could inhibit chondrocyte proliferation and promote apoptosis by targeting miR-34a. A previous study revealed that GAS5 overexpression played an important role in cell proliferation, apoptosis and growth (19). In radiation-induced thymic lymphoma, upregulation of GAS5 expression was involved in the regulation of cell proliferation and colony formation by participating in the chromosomal rearrangement of Notch1 (20). In non-small cell lung cancer, GAS5 could inhibit cell development by regulation of cell cycle progression via the p53/E2F1 signaling pathway, which acted as a tumor suppressor (21). Moreover, previous studies have revealed that GAS5 blockers accelerate cell proliferation and reduce

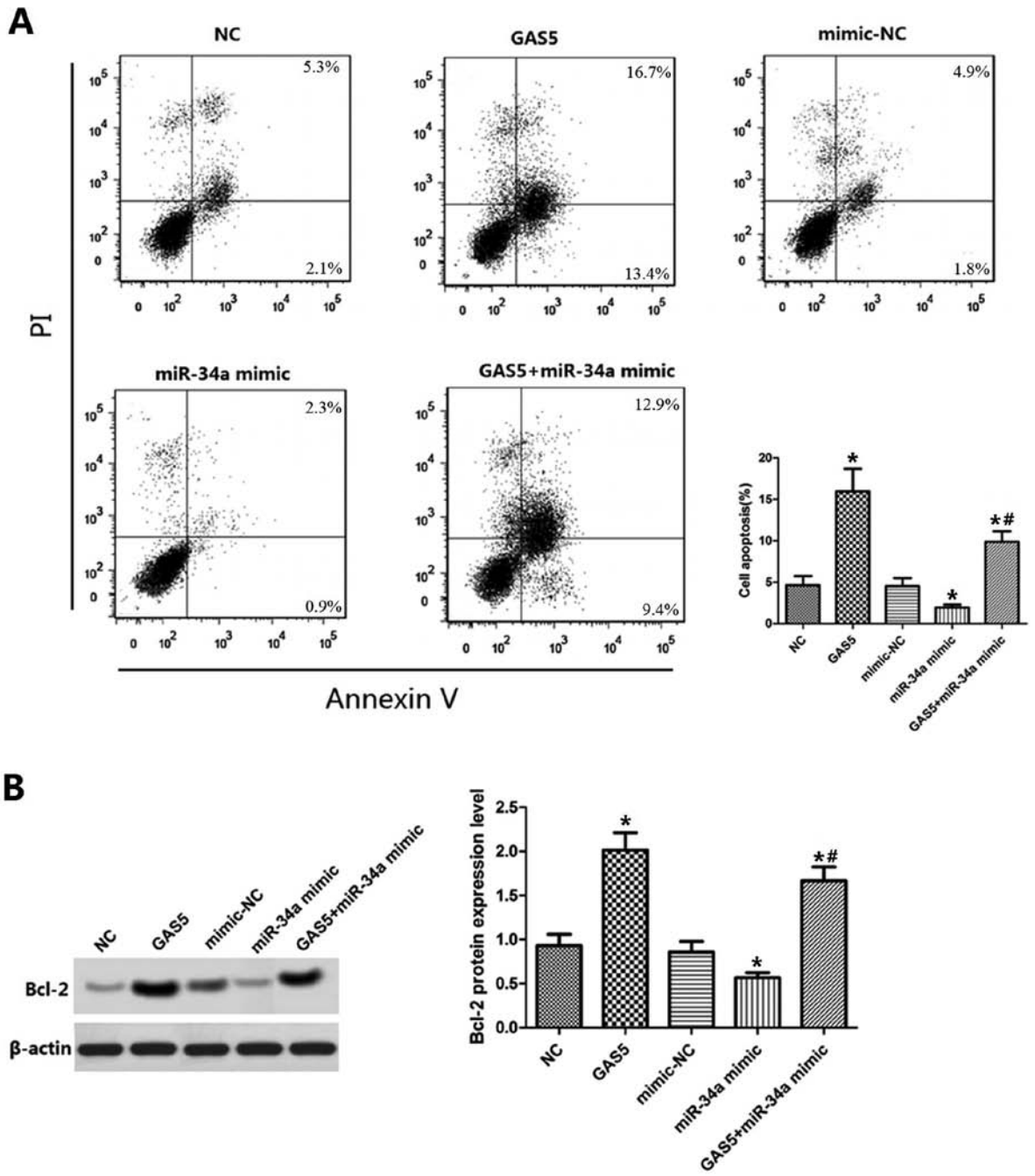

Figure 6. GAS5 targets miR-34a to regulate apoptosis. (A) Cell apoptosis by FCM. (B) Expression of Bcl-2 protein by western blotting. *P<0.05 vs. the NC group; #P<0.05 vs. the miR-34a mimic group. GAS5, growth arrest-specific transcript; FCM, flow cytometry.

apoptosis by promoting cell cycle progression (22). However, the role of GAS5 on OACs is not very clear. In the present study, differential expression of GAS5 in OACs and normal chondrocytes was observed. Silencing of GAS5 promoted cell proliferation and inhibited apoptosis, which revealed that GAS5 may be involved in the progression of OA by regulating OAC cell cycle progression.

The metabolic balance of the cartilage matrix guarantees the normal function of the cartilage tissue. Degradation of type II collagen in the ECM of chondrocytes can lead to abnormal cartilage morphology, as well as subchondral bone and trabecular bone structure (23). The direct cause of cartilage matrix degradation is mainly caused by increased protease activity of matrix metalloproteinases (MMPs) (24). Recent studies have revealed that lncRNAs play an important role in the ECM balance of chondrocytes, including lncRNA CIR (4,25) and MSR (26). In the present study, GAS5 silencing significantly inhibited the expression levels of MMP-13 in OACs. In addition, it increased the content of type II collagen and aggrecan in OACs, indicating that GAS5 may be involved in the development of OA by the regulation of chondrocyte ECM metabolism. OA is considered a degenerative and an inflammatory disease (12). Proinflammatory cytokines act on chondrocytes in order to cause chondrocyte metabolism and secretion disorders as well as chondrocyte proliferation and apoptosis abnormalities (27). Previous studies have suggested

that lncRNAs may be considered a bridge between inflammatory factors and cartilage destruction (2,28,29). Therefore, lncRNAs can be used as anti-inflammatory drugs for OA and can replace the use of glucocorticoid drugs, which exhibit several adverse reactions. The present study demonstrated that silencing of GAS5 reduced the levels of IL-6 and TNF-α in OACs, indicating that GAS5 may be associated with the regulation of several inflammatory factors in the osteoarthritic cartilage.

miR-34a expression is downregulated in various tumors, such as prostate cancer (30) and breast cancer (31), indicating tumor suppressor functions of this RNA. Previous studies have revealed that miR-34a can inhibit cell proliferation and promote cell apoptosis (32). *Bcl-2* is a target of miR-34a and are both involved in the regulation of apoptosis (33). In a previous study, bioinformatics analysis revealed binding of GAS5 and miR-34a and of miR-34a and *Bcl-2*, indicating that these molecules may be involved in the induction of OAC apoptosis. Moreover, luciferase reporter assays were used to test this hypothesis and it was revealed that GAS5 overexpression in OACs downregulated the expression of miR-34a, while it upregulated Bcl-2 levels in order to promote apoptosis induction. GAS5 and miR-34a were co-transfected in OACs to further validate the association between GAS5 and the miR-34a/Bcl-2 pathway. The results indicated that GAS5 overexpression could reverse the effects of miR-34a overexpression caused on the induction of apoptosis and on the expression of Bcl-2. Collectively, the results demonstrated that GAS5 could regulate the induction of OAC apoptosis by targeting the miR-34a/Bcl-2 axis.

In summary, the present study indicated that the expression levels of GAS5 were upregulated in OACs. GAS5 may participate in the development of OA by regulating apoptosis, cartilage ECM metabolism and chondrocyte inflammatory response. The mechanism of these processes may be associated with the regulation of the miR-34a/Bcl-2 pathway.

Acknowledgements

Not applicable.

Funding

No funding was received.

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

JY contributed to the conception and design of the present study, QJ contributed to the acquisition, analysis and interpretation of data, and drafted the manuscript. XQ contributed to the case collection, statistical analysis of clinical data and revised the manuscript critically for important intellectual content. YL and DW contributed to data collection and the performance of basic experiments. QJ contributed to the final manuscript revision and all authors agreed to be accountable for all aspects of the present study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by The Second Affiliated Hospital of Harbin Medical University Ethics Committee and all subjects signed the relevant informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Mandl LA: Osteoarthritis year in review 2018: Clinical. Osteoarthritis Cartilage 27: 359-364, 2019.
2. Zhang C, Wang P, Jiang P, Lv Y, Dong C, Dai X, Tan L and Wang Z: Upregulation of lncRNA HOTAIR contributes to IL-1β-induced MMP overexpression and chondrocytes apoptosis in temporomandibular joint osteoarthritis. Gene 586: 248-253, 2016.
3. Xing D, Liang JQ, Li Y, Lu J, Jia HB, Xu LY and Ma XL: Identification of long noncoding RNA associated with osteoarthritis in humans. Orthop Surg 6: 288-293, 2014.
4. Liu Q, Zhang X, Dai L, Hu X, Zhu J, Li L, Zhou C and Ao Y: Long noncoding RNA related to cartilage injury promotes chondrocyte extracellular matrix degradation in osteoarthritis. Arthritis Rheumatol 66: 969-978, 2014.
5. Kino T, Hurt DE, Ichijo T, Nader N and Chrousos GP: Noncoding RNA gas5 is a growth arrest- and starvation-associated repressor of the glucocorticoid receptor. Sci Signal 3: ra8, 2010.
6. Hu L, Ye H, Huang G, Luo F, Liu Y, Liu Y, Yang X, Shen J, Liu Q and Zhang J: Long noncoding RNA GAS5 suppresses the migration and invasion of hepatocellular carcinoma cells via miR-21. Tumour Biol 37: 2691-2702, 2016.
7. Yao T, Lu R, Zhang J, Fang X, Fan L, Huang C, Lin R and Lin Z: Growth arrest-specific 5 attenuates cisplatin-induced apoptosis in cervical cancer by regulating STAT3 signaling via miR-21. J Cell Physiol 234: 9605-9615, 2019.
8. Song J, Ahn C, Chun CH and Jin EJ: A long non-coding RNA, GAS5, plays a critical role in the regulation of miR-21 during osteoarthritis. J Orthop Res 32: 1628-1635, 2014.
9. 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.
10. Chen YY, Chen Y, Wang WC, Tang Q, Wu R, Zhu WH, Li D and Liao LL: Cyclin D1 regulates osteoarthritis chondrocyte apoptosis via WNT3/β-catenin signalling. Artif Cells Nanomed Biotechnol 47: 1971-1977, 2019.
11. Vedicherla S and Buckley CT: In vitro extracellular matrix accumulation of nasal and articular chondrocytes for intervertebral disc repair. Tissue Cell 49: 503-513, 2017.
12. Vila S: Inflammation in Osteoarthritis. P R Health Sci J 36: 123-129, 2017.
13. Niu J, Clancy M, Aliabadi P, Vasan R and Felson DT: Metabolic syndrome, its components, and knee osteoarthritis: The framingham osteoarthritis study. Arthritis Rheumatol 69: 1194-1203, 2017.
14. Hayashi S, Nishiyama T, Miura Y, Fujishiro T, Kanzaki N, Hashimoto S, Matsumoto T, Kurosaka M and Kuroda R: DcR3 induces cell proliferation through MAPK signaling in chondrocytes of osteoarthritis. Osteoarthritis Cartilage 19: 903-910, 2011.
15. Almonte-Becerril M, Navarro-Garcia F, Gonzalez-Robles A, Vega-Lopez MA, Lavalle C and Kouri JB: Cell death of chondrocytes is a combination between apoptosis and autophagy during the pathogenesis of Osteoarthritis within an experimental model. Apoptosis 15: 631-638, 2010.

16. Blanco FJ, Guitian R, Vazquez-Martul E, de Toro FJ and Galdo F: Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. Arthritis Rheum 41: 284-289, 1998.
17. Li Y, Li S, Luo Y, Liu Y and Yu N: LncRNA PVT1 regulates chondrocyte apoptosis in osteoarthritis by acting as a sponge for miR-488-3p. DNA Cell Biol 36: 571-580, 2017.
18. Zhang G, Wu Y, Xu D and Yan X: Long noncoding RNA UFC1 promotes proliferation of chondrocyte in osteoarthritis by acting as a sponge for miR-34a. DNa Cell Biol 35: 691-695, 2016.
19. Chen L, Yang W, Guo Y, Chen W, Zheng P, Zeng J and Tong W: Exosomal lncRNA GAS5 regulates the apoptosis of macrophages and vascular endothelial cells in atherosclerosis. PLoS One 12: e185406, 2017.
20. Mourtada-Maarabouni M and Williams GT: Role of GAS5 noncoding RNA in mediating the effects of rapamycin and its analogues on mantle cell lymphoma cells. Clin Lymphoma Myeloma Leuk 14: 468-473, 2014.
21. Shi X, Sun M, Liu H, Yao Y, Kong R, Chen F and Song Y: A critical role for the long non-coding RNA GAS5 in proliferation and apoptosis in non-small-cell lung cancer. Mol Carcinog 54 (Suppl 1): E1-E12, 2015.
22. Udayakumar T, Shareef MM, Diaz DA, Ahmed MM and Pollack A: The E2F1/Rb and p53/MDM2 pathways in DNA repair and apoptosis: Understanding the crosstalk to develop novel strategies for prostate cancer radiotherapy. Semin Radiat Oncol 20: 258-266, 2010.
23. Onuora S: Osteoarthritis: Cartilage matrix stiffness regulates chondrocyte metabolism and OA pathogenesis. Nat Rev Rheumatol 11: 504, 2015.
24. Tang LP, Ding JB, Liu ZH and Zhou GJ: LncRNA TUG1 promotes osteoarthritis-induced degradation of chondrocyte extracellular matrix via miR-195/MMP-13 axis. Eur Rev Med Pharmacol Sci 22: 8574-8581, 2018.
25. Li YF, Li SH, Liu Y and Luo YT: Long noncoding RNA CIR promotes chondrocyte extracellular matrix degradation in osteoarthritis by acting as a sponge for Mir-27b. Cell Physiol Biochem 43: 602-610, 2017.
26. Liu Q, Hu X, Zhang X, Dai L, Duan X, Zhou C and Ao Y: The TMSB4 pseudogene lncRNA functions as a competing endogenous RNA to promote cartilage degradation in human osteoarthritis. Mol Ther 24: 1726-1733, 2016.
27. Nixon AJ, Grol MW, Lang HM, Ruan MZC, Stone A, Begum L, Chen Y, Dawson B, Gannon F, Plutizki S, *et al*: Disease-modifying osteoarthritis treatment with interleukin-1 receptor antagonist gene therapy in small and large animal models. Arthritis Rheumatol 70: 1757-1768, 2018.
28. Kumar MM and Goyal R: LncRNA as a therapeutic target for angiogenesis. Curr Top Med Chem 17: 1750-1757, 2017.
29. Tong X, Gu PC, Xu SZ and Lin XJ: Long non-coding RNA-DANCR in human circulating monocytes: A potential biomarker associated with postmenopausal osteoporosis. Biosci Biotechnol Biochem 79: 732-737, 2015.
30. Yan X, Tang B, Chen B, Shan Y and Yang H; Reproducibility Project: Cancer Biology, Iorns E, Tsui R, Denis A, Perfito N and Errington TM: Replication Study: The microRNA miR-34a inhibits prostate cancer stem cells and metastasis by directly repressing CD44. Elife 8: pii: e43511, 2019.
31. He R, Liu P, Xie X, Zhou Y, Liao Q, Xiong W, Li X, Li G, Zeng Z and Tang H: circGFRA1 and GFRA1 act as ceRNAs in triple negative breast cancer by regulating miR-34a. J Exp Clin Cancer Res 36: 145, 2017.
32. Sun TY, Xie HJ, Li Z, Kong LF, Gou XN, Li DJ, Shi YJ and Ding YZ: miR-34a regulates HDAC1 expression to affect the proliferation and apoptosis of hepatocellular carcinoma. Am J Transl Res 9: 103-114, 2017.
33. Huang Q, Zheng Y, Ou Y, Xiong H, Yang H, Zhang Z, Chen S and Ye Y: miR-34a/Bcl-2 signaling pathway contributes to age-related hearing loss by modulating hair cell apoptosis. Neurosci Lett 661: 51-56, 2017.