hsa-miR-15a exerts protective effects against osteoarthritis by targeting aggrecanase-2 (ADAMTS5) in human chondrocytes

XIN LU, JIN LIN, JIN JIN, WENWEI QIAN and XISHENG WENG

Department of Orthopaedic Surgery, Peking Union Medical College Hospital, Beijing 100730, P.R. China

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Abstract. The aim of the present study was to examine the expression levels and role of hsa-miR-15a in osteoarthritis (OA), as well as the associated mechanisms. The expression levels of hsa-miR-15a and A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (ADAMTS5, also known as aggrecanase-2) were measured by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in both OA and normal chondrocytes. hsa-miR-21 mimics or antisense oligonucleotides (ASO) were co-transfected into the chondrocytes to examine the effects on the putative binding sites compared with the negative control (NC)-mimics or NC-ASO. The relative ADAMTS5 mRNA and protein levels were measured by RT-qPCR and western blot anlaysis, respectively. Moreover, after inhibiting the expression of hsa-miR-15a and ADAMTS5 by ASO and small interfering RNA (siRNA), respectively, the amounts of proteoglycan and collagen in the cellular matrix and medium were determined. Additionally, the expression levels of collagen II were measured by western blot analysis. hsa-miR-15a expression was downregulated, but ADAMTS5 expression was upregulated in the human OA chondrocytes compared to the normal chondrocytes. Luciferase reporter assay confirmed that the hsa-miR-15a binding site was in the ADAMTS5 gene 3'-untranslated region (3'-UTR), and ADAMTS5 was negatively regulated by hsa-miR-15a. The downregulation of hsa-miR-15a decreased the aggregation of proteoglycan and the collagen content, but increased the release of proteoglycan and collagen; total collagen production was significantly lower, and collagenase activity was markedly higher. The downregulation of ADAMTS5 increased the aggregation of proteoglycan and the collagen content, but decreased the release of proteoglycan and collagen, along with total collagen production. Moreover, collagenase activity was markedly lower. The findings of our study suggest that hsa-miR-15a exerts protective effects against OA by targeting ADAMTS5 in human chondrocytes.

Correspondence to: Dr Xin Lu, Department of Orthopaedic Surgery, Peking Union Medical College Hospital, 1 Shuai Fu Yuan, Wang Fu Jing Street, Beijing 100730, P.R. China
E-mail: luxin8899@126.com

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Introduction

Osteoarthritis (OA) is one of the most common degenerative joint disorders, and it affects elderly individuals in particular (1-3). It is characterized by the progressive loss of the cartilage matrix, a reduction in bone mass, the destruction of articular cartilage and osteophyte formation (4,5). The prevalence of this disease has been reported as continuing to increase, and this brings with it an enormous financial burden and related impairment of quality of life (6,7). However, to date, limited available disease-modifying treatment options for patients with OA are available due to the uncertain etiology of the disease (8). Currently, the only registered systemic oral drug therapy for OA is analgesics or anti-inflammatory agents to relieve symptoms (8,9).

The functional roles of microRNAs (miRNAs or miRs) in OA have increasingly attracted attention (10-12). miRNAs are short, small non-coding RNAs molecules that are involved in various human diseases (13,14). miRNAs regulate gene expression by repressing messenger RNA (mRNA) translation and promoting mRNA degradation (15). Previous studies have confirmed that a number of miRNAs, such as hsa-miR-21, hsa-miR-23a, hsa-miR-24, hsa-miR-27, hsa-miR-93, hsa-miR-100, hsa-miR-140, hsa-miR-145, hsa-miR-146a and hsa-miR-675 are associated with the development of OA (16-21). In addition, it has been previously demonstrated that A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (ADAMTS5, also known as aggrecanase-2) is involved in the development of OA (22). Of the ADAMTS family, ADAMTS4 and 5 are known to be the most efficient aggrecanases, and have been regarded as the most likely candidates for OA pathogenesis (23). Moreover, a study using animals indicated that the deletion of ADAMTS5 in mice with OA can prevent early aggrecan depletion and cartilage destruction (24). In addition, in another previous study, Matsukawa *et al* suggested that miR-125b regulates the expression of ADAMTS4 in OA chondrocytes (25).

However, to date, and to the best of our knowledge, there are limited studies available investigating the effects of hsa-miR-15a on OA, and the question of whether hsa-miR-15a regulates the expression of ADAMTS5 remains unanswered. Therefore, in the present study, we aimed to examine the effects of hsa-miR-15a on OA, as well as the associated mechanisms.

Materials and methods

Specimen selection. Femoral condyles or tibial plateaus were selected as specimens. Human OA cartilage was harvested from patients who underwent total knee replacement surgery (10 males and 6 females; mean age, 70±4 years) at the Department of Orthopaedic Surgery, Peking Union Medical College Hospital (Beijing, China). OA was diagnosed according to the clinical and radiological evaluation criteria published by the American College of Rheumatology (ACR) (26). In addition, none of the patients from whom the samples were collected had received intra-articular steroid therapy in the 3 months prior to the surgery. Healthy (control) articular cartilage was obtained from donors who had died (by car accident or due to cardiovascular and cerebrovascular diseases such as severe heart failure, or malignant tumors), within 12 h of death (8 males and 5 females; mean age, 67±5 years). The normal healthny individuals had no previous history of joint disorder. This study was approved by the Medical Ethics Committee of Peking Union Medical College Hospital (Beijing, China) and informed consent was acquired from all participants.

Cell culture and transient transfection. After collecting the cartilage specimens, the tissues were diced and digested in 1.5 mg/ml collagenase type 2 (CLS-2; Worthington, Lakewood, NJ, USA) in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) for 16 h at 37˚C. The DMEM solution was supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (all from Sigma, St. Louis, MO, USA). The isolated cells were then passed through a 100-μm cell strainer (Falcon; Becton-Dickinson Labware GmbH, Heidelberg, Germany), pelleted and washed with medium. The cultures were maintained in DMEM containing 10% FBS in a humidified incubator containing 5% CO_2 at 37˚C.

The cells were subsequently seeded in a 48-well prior to transfection. After post-culturing for 18-24 h, when the density of the chondrocytes had reached 70%, the cells were transfected using Lipofectamine 2000 (Invitrogen) according to a previously described method (27). In the present study, hsa-miR-15a mimics, pcDNA3/enhanced green fluorescent protein (EGFP)-ADAMTS5-3'-untranslated region (3'-UTR), antisense oligonucleotides (ASO)-hsa-miR-15a, ASO-NC (negative control), pcDNA3/EGFP-ADAMTS5-3'-UTR mutant and pDsRed2-N1, along with the plasmids were constructed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) based on standard protocols (28). Furthermore, ADAMTS5 expression was suppressed using the small interfering RNA (siRNA) synthesized by Sangon Biotech Co., Ltd. in both human OA and normal chondrocytes. A negative control (siNC) was also performed concurrently on each specimen. The sequence of the ADAMTS5 siRNA was 5'-AAGAUAAGCG CUUAAUGUCUU-3'. Cells were collected 48 h after transfection for subsequent assays.

Analysis of miRNA expression. After post-culturing for 24 h, total RNA was extracted from the cells using an miRNA Isolation kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. The relative expression of levels miRNAs (specific for hsa-miR-15a) were determined using TaqMan® MicroRNA assays (Applied Biosystems, Foster City, CA, USA) with the comparative $2^{-\Delta\Delta CT}$ method. Total RNA was reverse transcribed into complementary DNA (cDNA) using MuLV reverse transcriptase (Life Technologies, Carlsbad, CA, USA), followed by quantification using the QuantiTect SYBR-Green real-time-polymerase chain reaction kit (Qiagen SA, Hilden, Germany). Primers for miR-15a were designed and obtained from the TaqMan miRNA assays. U6 snRNA (Applied Biosystems) was used as a loading control for miRNA expression. The primer sequence for stem-loop RT-PCR hsa-miR-15a was 5'-CTCAACTGGTGTCGTGGAGTCGGC AATTCAGTTGAGCACAAACC-3'. The following primers were used for PCR: hsa-miR-15a forward, 5'-ACACT CCAGCTGGGTAGCAGCACATAATGG-3' and reverse, 5'-TGGTGTCGTGGAGTCG-3'; U6 forward, 5'-CTCGCTT CGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAA TTTGCGT-3'.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). We extracted total RNA from the chondrocytes using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The purity of the total RNA was assessed with the A260/A280 ratio, and values of 1.8 and 2.0 were considered good. Total RNA was reverse transcribed into cDNA and amplified, and was subsequently quantified using RT-qPCR with SYBR-Green Ex Taq on a LightCycler 480 (Roche Applied Science, Indianapolis, IN, USA). Primers for ADAMTS5 were designed and obtained from Invitrogen. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was used as a reference. The PCR conditions included 1 predenaturation cycle of 5 min at 95˚C, 40-50 cycles of 95˚C for 30 sec, 58-62˚C for 30 sec, and 72˚C for 30 sec, and a final extension at 72˚C for 5 min. Relative quantification was calculated using the $2^{-\Delta\Delta CT}$ method.

Bioinformatics analysis. Target genes of hsa-miR-15a were predicted by bioinformatics analysis using TargetScan 6.2 (http://www.targetscan.org) and/or microRNA.org (http://www. microrna.org).

Luciferase reporter assay. The 3'-UTR of ADAMTS5 was PCR-amplified, cloned into the psiCHECK-2 vector and co-transfected with the control or hsa-miR-15a precursor into the chondrocytes. After 48 h of transfection, luciferase activity was evaluated using the Dual-luciferase activity assay system (DLR; Promega, Madison, WI, USA).

Measurement of DNA content. Purified total DNA was quantified using Quant-iT™ PicoGreen® dsDNA reagent (Invitrogen) according to the manufacturer's instructions. Fluorescence was measured using a fluorescence microplate reader (Tecan Polarion, Stevenage, UK) at an excitation/emission wavelength of 480/520 nm. Lambda DNA (Sigma) was used as marker for determination of the DNA quantity.

Proteoglycan analysis. Total glycosaminoglycans (GAGs) were quantified using a 1,9-dimethylmethylene blue (DMMB) spectrophotometric analysis according to a previously described method (29). Briefly, following the addition of DMMB, the mixture was assayed for GAG at an absorbance of 525 nm. Chondroitin sulfate C (Sigma) was used as a reference.

Collagenase activity assay. After collecting the culture medium, collagenase activity assay was performed to determine the collagenase activity using an Enzchek Gelatinase/Collagenase assay kit (Invitrogen) according to the manufacturer's instructions. The assays were carried out in reaction buffer containing DQ Collagen Fluorescein conjugate at room temperature. Fluorescence was determined using a fluorescence microplate reader (SpectraMAX Gemini XS; Molecular Devices, Sunnyvale, CA, USA) at 485 nm excitation and 538 nm emission. Collagenase activity was determined using the formula provided with the assay kit and normalized to the weight of the explant. Collagenase type IV (*Clostridium*) was used as a reference standard.

Western blot analysis. Following culture for 24 h, cell protein was extracted using protein lysis buffer. Protein concentration was assessed using the Bio-Rad DC protein assay kit (Bio-Rad, Ivry-sur-Seine, France). The protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and was then transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). The membranes were blocked in a sealed in 5% fresh non-fat dry milk for 2 h and were then incubated with the primary anti-type II collagen antibody (1:1,000 dilution; MAB1330; Chemicon, Millipore, Billerica, CA, USA) and primary anti-ADAMTS5 antibody (1:100 dilution; ab45042; Abcam, Cambridge, UK) for 2 h at room temperature or overnight at 4˚C, followed with horseradish peroxidase-conjugated anti-mouse secondary antibody (accession numbers ab195239). An anti-GAPDH antibody (sc-365062; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) was used as a loading control. Finally, the samples were tested using enhanced chemiluminescence and densitometric analysis. For relative expression analysis, NIH Image J software (NIH Image, Bethesda, MD, USA) was performed to determine band intensity.

Statistical analysis. In the present study, the statistical package for the social sciences (SPSS) (version 17.0; SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. A student's t-test was performed to study statistical comparisons. A p-value <0.05 was considered to indicate a statistically significant difference.

Results

Expression of hsa-miR-15a is decreased in human OA chondrocytes. To dertermine the expression levels of hsa-miR-15a in human OA chondrocytes, we determined the levels of hsa-miR-15a in human OA cartilage specimens and normal cartilage tissues by RT-qPCR. As shown in Fig. 1, the expression level of hsa-miR-15a was significantly decreased in the OA chondrocytes compared with the normal cartilage tissue (P<0.05).

ADAMTS5 gene 3'-UTR carries a putative hsa-miR-15a binding site and is negatively regulated by hsa-miR-15a. To predict whether the ADAMTS5 gene mRNA 3'-UTR contains a hsa-miR-15a binding site, TargetScan 6.2 and microRNA.org were used to confirm the prediction. The results revealed that a binding site for hsa-miR-15a was in the ADAMTS5 mRNA 3'-UTR (Fig. 2A).

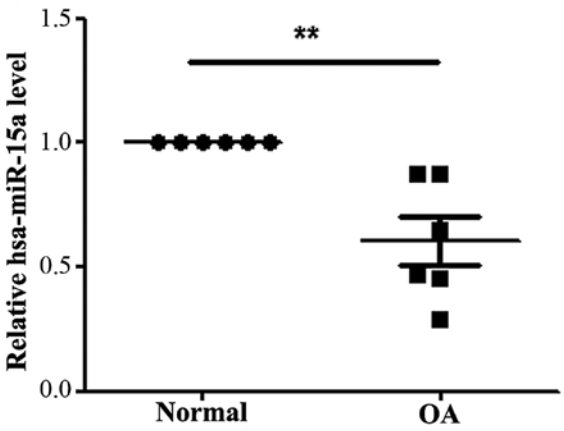

Figure 1. mRNA expression level of hsa-miR-15a in human osteoarthritic (OA) chondrocytes and normal chondrocytes. **p<0.01 compared with the normal control group.

In order to confirm that hsa-miR-15a binds to this region and leads to translational repression, we cloned the putative binding site into the pcDNA3/EGFP plasmid downstream of the EGFP reporter construct and co-transfected this plasmid into the cells with ASO-hsa-miR-15a or ASO-negative control (NC). We found that the relative EGFP intensity was significantly higher in the cells that were co-transfected with ASO-miR-15a compared with the cells co-transfected with ASO-NC. In addition, we cloned the putative binding site into pcDNA3/EGFP downstream of the EGFP reporter construct and co-transfected this plasmid with hsa-miR-15a mimics or NC. The results revealed that the relative EGFP intensity was significantly lower in the cells co-transfected with hsa-miR-15a mimics compared with those co-transfected with NC (Fig. 2B). Additionally, we performed RT-qPCR and western blot analysis to measure the mRNA and protein expression levels of ADAMTS5, respectively. Both RT-qPCR (Fig. 2C) and western blot analysis (Fig. 2D and E) revealed that the ADAMTS5 expression levels were significantly lower in the hsa-miR-15a mimics-transfected group compared with the NC-transfected group (P<0.05); however these levels were significantly higher in the ASO-miR-15a-transfected group compared with the ASO-NC-transfected group (P<0.05), respectively. These results suggest that hsa-miR-15a binds directly to the 3'-UTR of ADAMTS5 mRNA and inhibits gene expression. These results also indicate that ADAMTS5 is a direct target of hsa-miR-15a.

Downregulation of hsa-miR-15a expression decreases the aggregation of proteoglycan and increases the release of proteoglycan. The amount of proteoglycans was significantly decreased in the cell substrate generated by the OA chondrocytes transfected with ASO-miR-15a compared to the ASO-NC-transfected group (Fig. 3A), while the amount of proteoglycans was significantly increased in the culture medium compared to the ASO-NC-transfected group (Fig. 3B). However, as regards the total amount of proteoglycans, there were no significant differences observed between the ASO-miR-15a-transfected group and the controls (ASO-NC) (Fig. 3C). The total amount of proteoglycans was measured by combining the amount found in the cell substrate and that released into the medium at the end of the culture period.

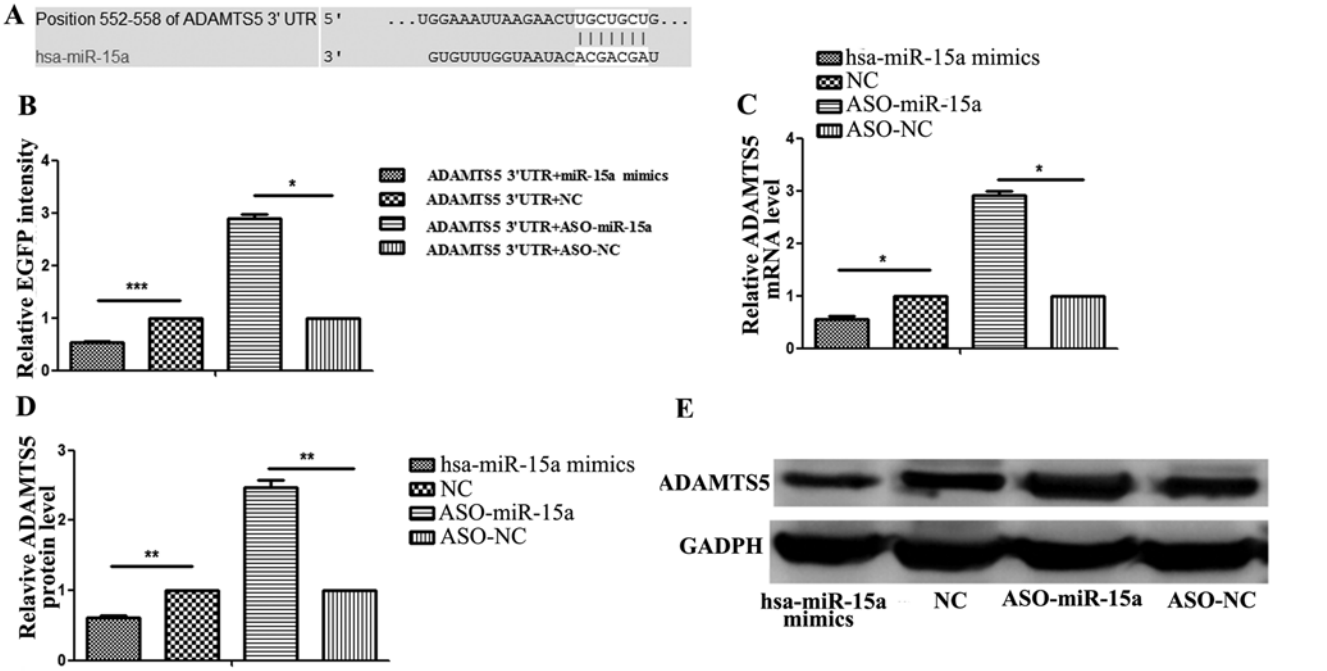

Figure 2. A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (ADAMTS5, also known as aggrecanase-2) gene 3'-UTR carries a putative hsa-miR-15a binding site and is negatively regulated by hsa-miR-15a. (A) Bioinformatics analysis predicted that ADAMTS5 is the target gene of hsa-miR-15a. (B) Luciferase reporter vector confirmed that hsa-miR-15a may bind directly to the 3'-UTR of ADAMTS5 mRNA. (C) Relative ADAMTS5 mRNA expression level regulated by hsa-miR-15a. (D) Relative ADAMTS5 protein expression level regulated by hsa-miR-15a. (E) Western blot analysis of ADAMTS5 expression regulated by hsa-miR-15a. ASO, antisense oligonucleotides; NC, negative control. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ compared with the corresponding control group.

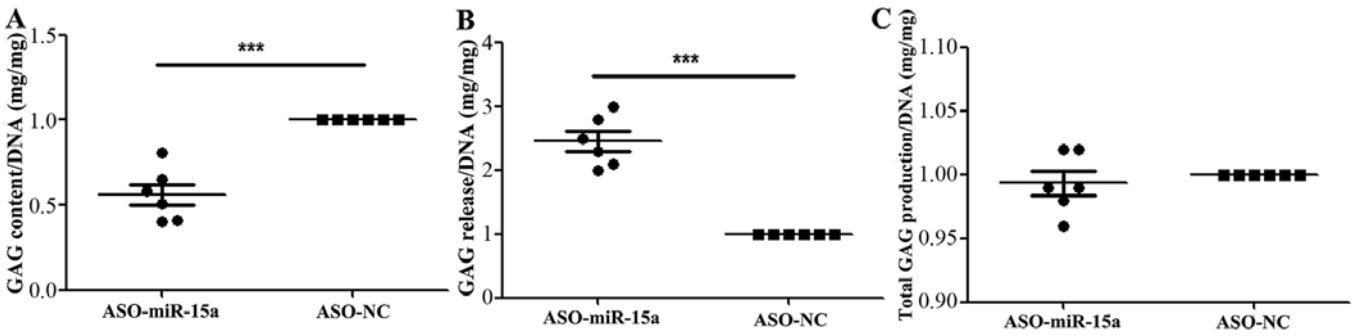

Figure 3. Low expression of hsa-miR-15a increases the release of proteoglycan and decreases the aggregation of proteoglycan. (A) Glycosaminoglycan (GAG) content in cell substrate is regulated by hsa-miR-15a. (B) GAG content in medium is regulated by hsa-miR-15a. (C) Total amount of GAG is regulated by hsa-miR-15a. ASO, antisense oligonucleotides; NC, negative control. $^{***}p<0.001$ compared with the ASO-NC group.

Downregulation of hsa-miR-15a expression decreases the aggregation of collagen and increases the release of collagen. The amount of collagen was significantly decreased in the cell substrate generated by the OA chondrocytes transfected with ASO-miR-15a compared to the ASO-NC-transfected group (Fig. 4A), while the amount of collagen was significantly increased in the culture medium of the cells transfected with ASO-miR-15a compared to the ASO-NC-transfected group (Fig. 4B). In addition, the total amount of collagen was significantly decreased in the ASO-miR-15a-transfected group compared with the controls (ASO-NC) (Fig. 4C). However, collagenase activity was significantly increased in ASO-miR-15a-transfected group compared with the controls (Fig. 4D). Moreover, the protein expression of collagen II was significantly lower (0.75 ± 0.14 vs. 1.88 ± 0.16, with a 40% decrease) in the ASO-miR-15a-transfected group compared with the ASO-NC-transfected group (Fig. 4E). These results indicate that the decreased expression of hsa-miR-15a inhibits the aggregation of collagen, while the overexpression of hsa-miR-15a promotes the aggregation of collagen.

Downregulation of ADAMTS5 increases the aggregation of proteoglycan and decreases the release of proteoglycan. We performed RT-qPCR to measure the expression levels of ADAMTS5 in human OA chondrocytes, as well as in normal cartilage tissue. As shown in Fig. 5A, the expression level of ADAMTS5 was significantly increased in the OA chondrocytes compared with the normal cartilage tissue (P<0.05).

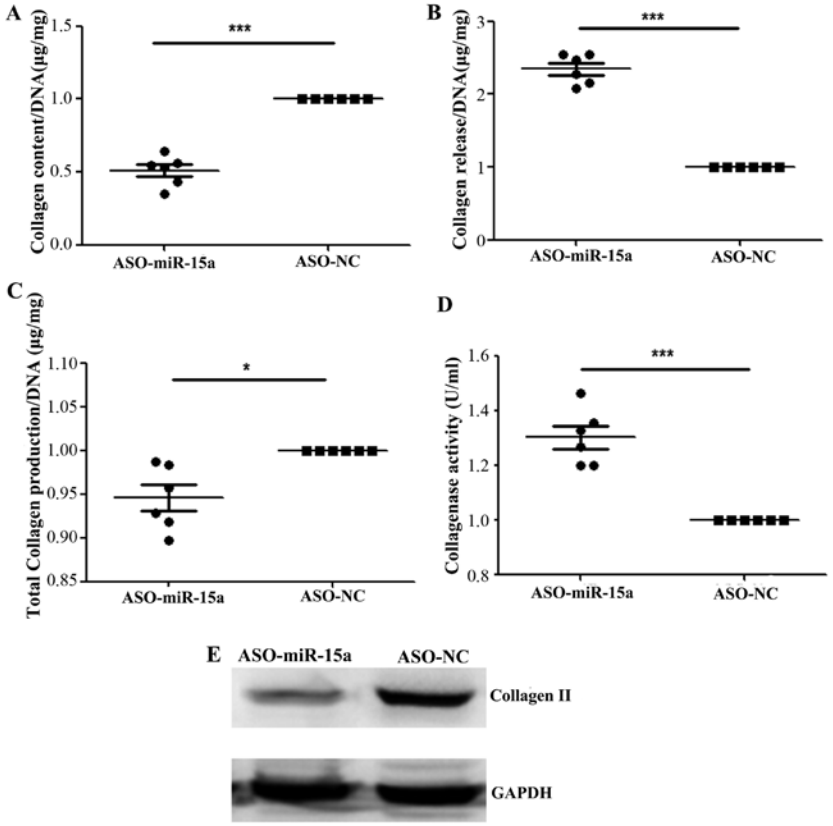

Figure 4. Low expression of hsa-miR-15a increases the release of collagen and decreases the aggregation of collagen. (A) Collagen content in cell substrate is regulated by hsa-miR-15a. (B) Collagen content in medium is regulated by hsa-miR-15a. (C) Total amount of collagen is regulated by hsa-miR-15a. (D) Collagenase activity is regulated by hsa-miR-15a. (E) Western blot analysis of collagen II regulated by hsa-miR-15a. ASO, antisense oligonucleotides; NC, negative control. *p<0.05 and ***p<0.001 compared with the ASO-NC group.

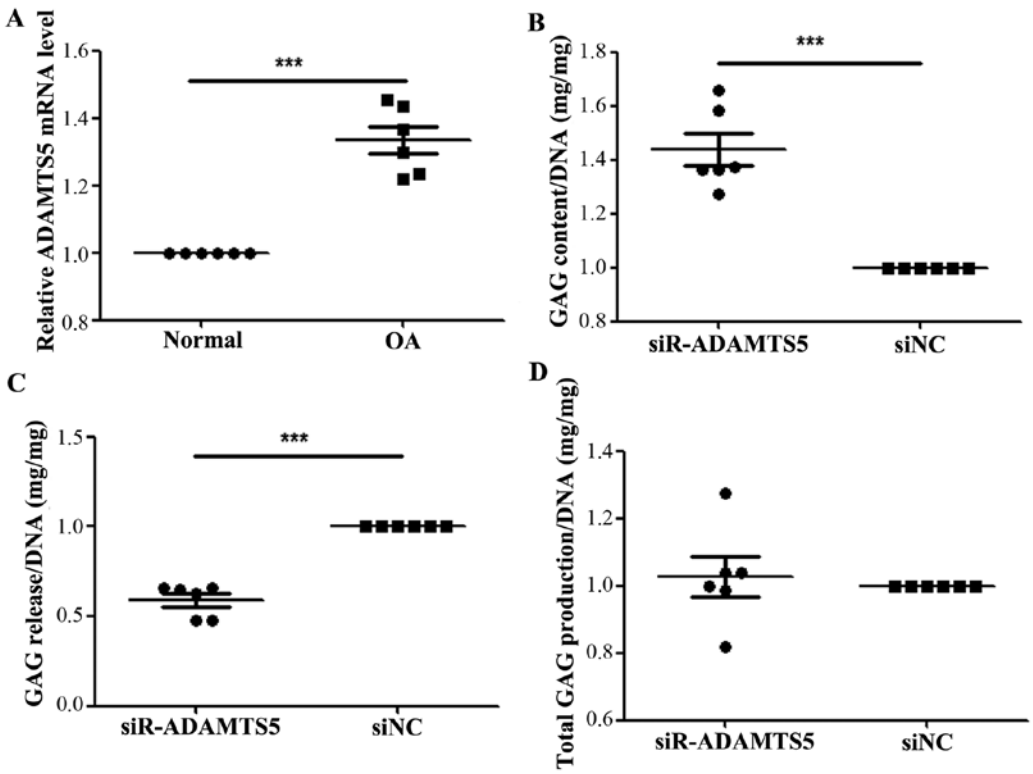

Figure 5. A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (ADAMTS5, also known as aggrecanase-2) decreases the aggregation of proteoglycan and increases the release of proteoglycan. (A) Relative ADAMTS5 mRNA expression in human osteoarthritic (OA) chondrocytes and normal chondrocytes. (B) Glycosaminoglycan (GAG) content in cell substrate is regulated by ADAMTS5. (C) GAG content in medium is regulated by ADAMTS5. (D) Total amount of is GAG regulated by ADAMTS5. ASO, antisense oligonucleotides; NC, negative control. ***p<0.001 compared with the siNC group.

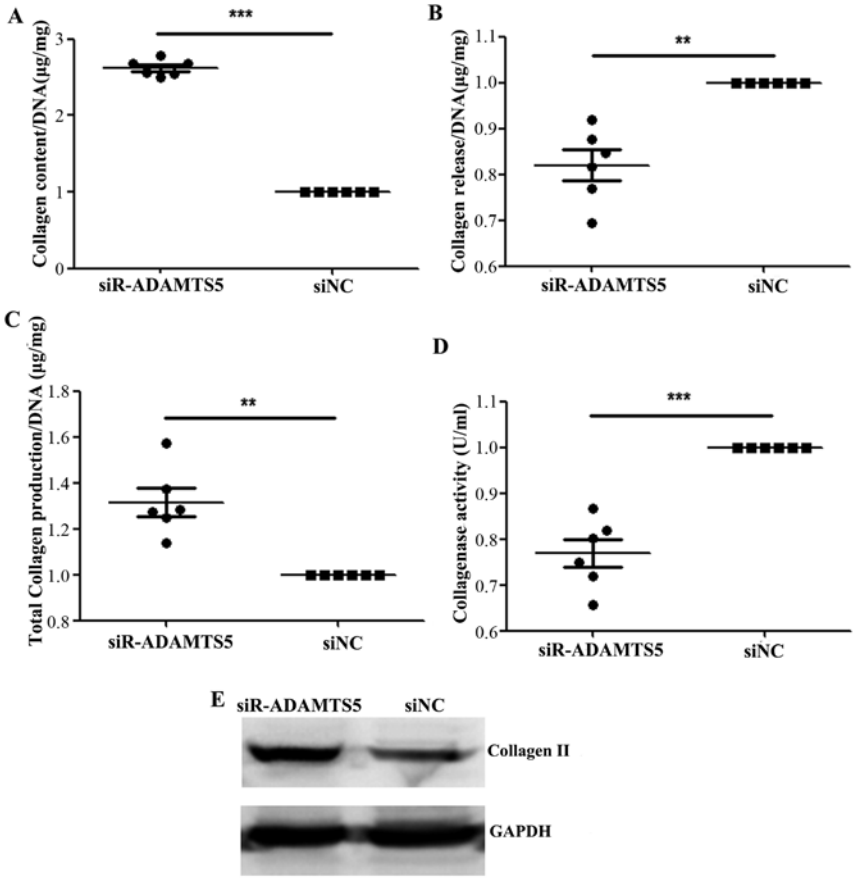

Figure 6. A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 5 (ADAMTS5, also known as aggrecanase-2) decreases the aggregation of collagen and increases the release of collagen. (A) Collagen content in cell substrate is regulated by ADAMTS5. (B) Collagen content in medium is regulated by ADAMTS5. (C) Total amount of collagen is regulated by ADAMTS5. (D) Collagenase activity is regulated by ADAMTS5. (E) Western blot analysis of collagen II regulated by ADAMTS5. ASO, antisense oligonucleotides; NC, negative control. $^{**}p<0.01$ and $^{***}p<0.001$ compared with the siNC group.

To confirm a more definitive function of ADAMTS5 in the development of OA, we used ADAMTS5 siRNA to transfect human OA chondrocytes. The results revealed that the amount of proteoglycan was significantly increased in the chondrocyte substrate of the siR-ADAMTS5 group compared with that of the silenced negative control (siNC) group (Fig. 5B), whereas the amount released into the medium was significantly decreased compared with siNC group (Fig. 5C). No significant differences were observed in the total amount of proteoglycans between the 2 groups (Fig. 5D).

Downregulation of ADAMTS5 increases the aggregation of collagen and decreases the release of collagen. The amount of collagen was significantly increased in the chondrocyte substrate of the siR-ADAMTS5 group compared with that of the siNC group (Fig. 6A), whereas the amount released into the medium was significantly decreased compared with the siNC group (Fig. 6B). In addition, the total amount of collagen was significantly increased in the siR-ADAMTS5 group compared with the siNC group (Fig. 6C), while the collagenase activity was significantly lower in the siR-ADAMTS5 group than that in the siNC group (Fig. 6D). Furthermore, the protein expression of collagen II was significantly higher (2.22 ± 0.04 vs. 1.04 ± 0.02, with a 50% increase) in the siR-ADAMTS5 group compared with the siNC group (Fig. 6E).

Discussion

miRNAs are involved in normal skeletal development (30), and their levels are associated with the pathogenesis of degenerative disorders, such as OA (31,32). Hence, it is necessary to elucidate the function of miRNA in the OA. In the present study, we examined the expression and role of hsa-miR-15a in the pathogenesis of OA. We found that hsa-miR-15a was expressed at lower levels in OA chondrocytes compared with normal healthy control tissue, suggesting that hsa-miR-15a plays an important role in the pathogenesis of OA. Using TargetScan6.2 and microRNA.org to screen the targets of hsa-miR-15, ADAMTS5 was predicted to be a target of hsa-miR-15a, and the binding site was found in the ADAMTS5 gene 3'-UTR. Moreover, the expression of ADAMTS5 was found to be negatively regulated by hsa-miR-15a. In addition, ADAMTS5, as a key enzyme in OA, has been reported to play a role in the degradation of articular cartilage (24). Thus, we hypothesized that hsa-miR-15a may exert protective effects against OA through the negative regulation of the expression of ADAMTS5.

In order to prove this hypothesis, we first determined the expression level of has-miR-15a in both OA and normal chondrocytes. As previously mentioned, the expression level of hsa-miR-15a was significantly decreased in the OA chondrocytes compared with the normal chondrocytes.

Subsequently, we further assessed the prediction revealed by bioinformatics analysis that ADAMTS5 is targeted by hsa-miR-15a. Luciferase reporter assay confirmed the prediction, and demonstrated that ADAMTS5 is directly targeted by hsa-miR-15a at its 3'-UTR. In addition, we also determined the expression mRNA and protein levels of ADAMTS5 when the level of hsa-miR-15a had been altered. By examining the overexpression (by transfection with hsa-miR-15a mimics) or downregulation (by transfection with ASO-hsa-miR-15a) of the level of hsa-miR-15a, we found that the expression level of ADAMTS5 inversely correlated with hsa-miR-15a.

As a key enzyme in OA, in this study, ADAMTS5 was found to be markedly increased in OA chondrocytes, which was in line with the findings of previous studies (22,33,34). ADAMTS5 (namely aggrecanase-2) are members of the ADAMTS family. Together with ADAMTS4 (namely aggrecanase-1), they have the ability to cleave the aggrecan core protein (35,36), resulting in the destruction of cartilage function, which is subsequently followed by irreversible collagen degradation (22,37,38). Previous research has confirmed that ADAMTS4 and ADAMTS5 are the most efficient aggrecanases *in vitro*, but ADAMTS5 presents more actively than ADAMTS4, and rather than ADAMTS4, ADAMTS5 participates in the early stages of development of OA (33,39). Moreover, the deletion of ADAMTS5 protects against OA by decreasing aggrecan degradation (40). Additionally, the intervention of aggrecanases has been considered as an effective therapeutic method for treatment of OA (37). In the present study, we demonstrated the important role of ADAMTS5 in the pathogenesis of OA. To confirm the function of ADAMTS5, we further silenced the expression of ADAMTS5. Our results indicated that the aggregation of proteoglycan and the collagen content were markedly increased, but the release of proteoglycan and collagen was significantly decreased following the suppression of ADAMTS5. Moreover, the total collagen production was significantly higher, and collagenase activity was markedly lower in the siR-ADAMTS5-transfected group compared with the siNC-transfected group. However, the silencing of ADAMTS5 did not have a marked effect on the total proteoglycan production, indicating that diminished breakdown induced the increased retention of proteoglycan content, rather than the synthetic activity of proteoglycan being increased. These results are in accordance with those of a previous study conducted by Vonk *et al* (41), which suggests that ADAMTS5 plays an important role in the pathogenesis of OA through the inhibition of the aggregation of proteoglycan and collagen content and the increase in the collagenase activity.

The expression of hsa-miR-15a was downregulated in this study to confirm the protective effects of hsa-miR-15a against OA. Opposite effects were observed in the amount of proteoglycan and collagen in the cellular matrix and medium, the expression level of collagen II, and collagenase activity compared with the effects revealed by the suppression of ADAMTS5 expression. Our results demonstrated that the aggregation of proteoglycan and collagen content were significantly decreased, but the release of proteoglycan and collagen was markedly increased in the ASO-miR-15a-transfected compared with the normal group (ASO-NC). Moreover, the total collagen production was significantly lower, and collagenase activity was markedly higher following the downregulation of miR-15a. These data suggest that hsa-miR-15a exerts protective effects against OA by suppressing the expression of ADAMTS5.

In conclusion, to the best of our knowledge, this study provides the first evidence that hsa-miR-15 plays a protective role in OA by directly targeting ADAMTS5. Our results provide significant clinical evidence for future studies investigating the beneficial effects of targeting ADAMTS5 as a biological method for the treatment of OA.

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