miR-195 inhibits the proliferation and migration of chondrocytes by targeting GIT1

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Abstract. Previous studies have demonstrated that G-protein coupled receptor kinase interacting protein-1 (GIT1) and microRNAs (miRNAs) serve an important role in chondrocyte proliferation and migration. However, a limited number of studies conducted thus far have investigated the association between GIT1 and miRNAs. In the present study, putative miR-195 binding sites in the GIT1 3'-untranslated region were identified using common bioinformatic algorithms (miRanda, TargetScan, miRBase and miRWalk), and it was demonstrated that they may be involved in regulating GIT1 expression. Following transfection of miR-195 mimics in chondrocytes, the expression of GIT1 was significantly reduced, whereas the expression was significantly increased following transfection with miR-195 inhibitors. In addition, the results of the current study demonstrated that increased miR-195 expression may downregulate chondrocyte proliferation and reduce cell migration. However, chondrocyte proliferation and migration was enhanced following suppression of miR-195 expression. Furthermore, upon co-transfection of miR-195 and GIT1 expression vectors, the inhibitory effect of miR-195 on chondrocyte proliferation and migration was attenuated. Therefore, miR-195 may affect chondrocyte proliferation and migration via targeted regulation of GIT1 expression. The results of the current study provide novel evidence for the regulatory mechanisms of miRNAs in bone and cartilage tissues, which may facilitate further research and provide a greater understanding of different osteoarticular diseases.

Introduction

Chondrocyte differentiation, proliferation, secretion and apoptosis are considered to be critical in bone development and for the maintenance of joint function (1). In the bone microenvironment, a number of growth factors are involved in regulating chondrocyte metabolism (2).

G-protein-coupled receptor kinase interacting protein-1 (GIT1) is considered to serve a vital function in bone development and growth (3-6). Menon *et al* (3) demonstrated that GIT1 may function as a key regulator of bone mass *in vivo* by regulating osteoclast function, and suggested that it may be a potential target for osteoporosis therapy. GIT1, a protein-binding partner of G protein-coupled receptor kinase 2, was discovered by a yeast two-hybrid technique (7) and is constitutively expressed in mammals and birds. GIT1 is primarily located at focal adhesion points and at cytoplasmic structures within cells, such as inclusion bodies (8). The function of GIT1 was initially determined to involve regulating the function of cell surface G-protein coupled receptors in cells (9). However, a recent study has demonstrated that GIT1 may serve a role in regulating cytoskeletal dynamics during cell growth and migration processes (10). In particular, GIT1 binds to a number of cytoskeletal proteins, such as paxillin and focal adhesion kinase, and is regulated by Src to promote cell migration (10).

MicroRNAs (miRNAs) have been a focus of research in the field of osteoarticular disease (11). An increasing number of studies have indicated that miRNAs serve an important role in regulating cell differentiation and extracellular matrix secretion in bone and chondroid tissue generation and metabolism processes, and are involved in the regulation of multiple signaling pathways in the bone and joint tissues (12,13). For example, miR-140 is specifically expressed in the cartilage of mouse embryos during long and flat bone development, and can suppress histone deacetylase 4 expression to maintain the chondrocyte phenotype (14). Kim *et al* (15) indicated that miR-221 may regulate cell proliferation by negatively regulating mouse double minute 2 homolog, thereby inhibiting Slug degradation during the chondrogenesis of limb mesenchymal cells.

To date, a limited number of studies have investigated miRNAs that target GIT1, and no research conducted thus far has investigated GIT1 and its associated miRNAs in chondrocytes. Therefore, the aim of the present study was to investigate the interaction between GIT1 and miRNAs in chondrocytes.

Materials and methods

Cell culture. The CHON-002 human chondrocyte cell line was purchased from the American Type Culture Collection

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(Manassas, VA, USA), and cultured in Dulbecco's Modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% heat-inactivated fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.) and 0.1 mg/ml G-418 solution (Sigma-Aldrich; Merck-Millipore, Darmstadt, Germany).

Expression vector construction and transfection. TRIzol reagent (Thermo Fisher Scientific, Inc.) was used to extract RNA from chondrocytes ($1x10^7$ cells), according to the manufacturer's instructions. This was followed by reverse transcription-polymerase chain reaction (RT-PCR) to amplify the coding region of GIT1. The primers used for GIT1 amplification are listed in Table I. The product was digested with *Kpn*I and *Eco*RI restriction endonucleases (Takara Biotechnology Co., Ltd., Dalian, China), and then cloned into pcDNA3.1 vectors (Thermo Fisher Scientific, Inc.), sequenced and verified in a 3730xl DNA Analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Cells ($1x10^5$) were seeded in 6-well culture plates and cultured until they reached ~70% confluence, before expression vectors were transfected using Lipofectamine 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The concentration of the GIT1 transfection vector used was 4 μg/well. The GIT1 small-interfering RNA (siRNA; cat. no. sc-35477; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), miR-195 mimics and inhibitors (Shanghai GenePharma Co., Ltd., Shanghai, China) were all transfected into cells at a concentration of 50 nM/well.

RT-quantitative PCR (RT-qPCR). Total RNA was isolated from cultured cell samples using TRIzol reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions, and the mirVana miRNA Isolation kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used to purify miRNAs. Target gene expression levels of GIT1 and miR-195 were measured using RT-qPCR. cDNA was synthesized by reverse transcription using random and oligo-dT primers (Promega Corporation, Madison, WI, USA) or specific primers for miRNA-195 (Takara Biotechnology Co., Ltd.), together with the GoScript Reverse Transcription System (Promega Corporation). qPCR was performed using the GoTaq qPCR Master Mix (Promega Corporation) and an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The thermocycling conditions were as follows: 95˚C for 2 min; and 40 cycles of 95˚C for 15 sec and 60˚C for 32 sec. The primer sequences are shown in Table I. GAPDH served as a control for GIT1 expression and U6 as a control for miR-195. To measure miRNA expression, specific primers for miRNA-195 and U6 were used. Three independent experiments were conducted for each sample. Data were analyzed by comparing the $2^{-\Delta\Delta Cq}$ values (16).

Western blot analysis. Total protein was extracted by incubating cells ($1x10^6$) in radioimmunoprecipitation assay (RIPA) buffer (cat. no. sc-24948; Santa Cruz Biotechnology, Inc.) for 10 min in an ice bath, prior to loading of 50 μg samples in 10% SDS-PAGE gels for separation and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Membranes were blocked with 5% non-fat milk for 1 h at room temperature and then incubated with antibodies against GIT1 (cat. no. 2919; dilution, 1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA) or GAPDH (cat. no. 2118; 1:1,000; Cell Signaling Technology, Inc.) in 5% non-fat milk overnight at 4˚C. Immunoreactive proteins were visualized using incubation with horseradish peroxidase-conjugated IgG secondary antibodies (cat. no. 7074; dilution, 1:7,000-8,000; Cell Signaling Technology, Inc.) at room temperature for 1 h and enhanced chemiluminescence reagents (Pierce; Thermo Fisher Scientific, Inc.). Images were analyzed using Image-Pro Plus 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). Each band was scanned with background correction, and values were expressed as the mean ± standard deviation.

Dual luciferase assay. The software applications miRanda (http://www.microrna.org/microrna/getGeneForm.do), TargetScanHuman (http://www.targetscan.org/vert_71/), miRBase (http://www.mirbase.org/) and miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/) predicted that GIT1 has miR-195a-3p binding sites. The GIT1 3'-UTR was cloned into the psiCHECK-2 vector (Promega Corporation), and the seed region of the miR-195 binding site in the 3'-UTR was mutated using the QuikChange II Site-Directed Mutagenesis kit (Agilent Technologies, Inc., Santa Clara, CA, USA). For the luciferase assay, $2x10^4$ HEK293A cells (American Type Culture Collection) were seeded in 24-well dishes and were cultured until they reached 80% confluence. Cells were transfected with psiCHECK-2 (containing the wild-type GIT1 3'-UTR or the mutated form) together with miR-195 mimics using the Lipofectamine 2000 transfection agent (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were analyzed at 24 h post-transfection. Firefly and *Renilla* luciferase activities were quantified in cell lysates using the Dual-Luciferase Reporter assay kit (Promega Corporation) on a Glomax 20/20 luminometer (Promega Corporation) according to the manufacturer's instructions. Luciferase readings were corrected to background readings and firefly luciferase values were normalized to *Renilla* values in order to determine the transfection efficiency. Samples were analyzed in triplicate and three independent experiments were conducted.

Immunoprecipitation. A total of $1x10^7$ HEK 293A cells were lysed in RIPA buffer containing 1 mM protease inhibitors (Roche Diagnostics GmbH, Mannheim, Germany) and centrifuged at 10,000 x *g* for 10 min at 4˚C. For immunoprecipitation, 5 ml of supernatant from monoclonal anti-Ago2 antibody (cat. no. 2897; dilution, 1:50; Cell Signaling Technology, Inc.) was coupled to 80 μl Protein-G-Sepharose beads (GE Healthcare Life Sciences, Chalfont, UK). Beads were subsequently incubated with 10 ml HEK 293 lysate for 5 h under constant rotation at 4˚C. Following incubation, the beads were washed three times with Tris-buffered saline. The beads were then washed once with phosphate-buffered saline (PBS). Co-immunoprecipitated RNA was extracted using phenol: chloroform: isoamyl alcohol (25:24:1; cat. no, 15593-031; Invitrogen; Thermo Fisher Scientific, Inc.). The RNA pellet was used for RT-qPCR analysis of GIT1 expression, using the aforementioned methods.

Table I. Primer sequences used for the purposes of the present study.

Analysis	Name	Sense primer sequence (5'-3')	Antisense primer sequence (5'-3')	Product size (bp)
GIT1 CDS amplification	GIT1 CDS	GGGGTACCGCCACCATGTC CCGAAAGGGGCCG	CGGAATTCTCACTGCTTCTTCT CTCGGGTG	
RT-qPCR	GAPDH	GGTATCGTGGAAGGACTC	GTAGAGGCAGGGATGATG	128
	GIT1	ATGTATGAACCTGGCTCTG	TGAATAGATGGCGTCGTC	114
	miR-195 RT	CTCAACTGGTGTCGTGGAGTCG GCAATTCAGTTGAGGCCAATAT		
	miR-195	ACACTCCAGCTGGGTAGC AGCACAGAAATATT	CTCAACTGGTGTCGTGGA	71
	U6 RT	AACGCTTCACGAATTTGCGT		
	U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT	94
GIT1 3'-UTR amplification	GIT1 3'-UTR	CCG**CTCGAG**CCTCTCTC CCCACACCCTCA	ATAAGAAT**GCGGCCGC**TA ACAGCTCATGGTCACTTCTTTAT	

Bold and underlined bases indicate the restriction enzyme cutting site. RT, reverse transcription; qPCR, quantitative polymerase chain reaction; GIT1, G-protein-coupled receptor kinase interacting protein-1; CDS, coding sequence; UTR, untranslated region.

BrdU cell proliferation assay. A BrdU assay was used to investigate the roles of miR-195 and GIT1 on cell proliferation. Briefly, the cultured cells ($1x10^5$ chondrocytes) were seeded into 6-well plates and incubated for 24 h before miR-195 mimics and inhibitors, plasmids or siRNAs were transfected into cells using the Lipofectamine 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.). A BrdU Cell Proliferation Assay kit (Cell Signaling Technology, Inc.) was used to determine cell proliferation according to the manufacturer's instructions. Following transfection and incubation for 48 h, the medium was removed and cells were labeled with 10 mM BrdU for 3 h at 37˚C. Cells were fixed and incubated with peroxidase-conjugated anti-BrdU antibody for 90 min at room temperature. Subsequently, the peroxidase substrate (3,3',5,5'-tetramethylbenzidine) was added, and BrdU incorporation was quantitated by differences in absorbance at wavelength of 370 subtract wavelength of 492 nm. Cell proliferation was expressed as the mean percentage relative to the control values (set at 100%).

Cell migration examination. Cell migration rates were measured using a transwell chamber (BD Biosciences, Franklin Lakes, NJ, USA) containing Matrigel. The trypsinized chondrocytes were diluted to a final concentration of $2x10^6$ cells/ml in serum-free media, and 100 μl cell suspension was added into the upper chamber and 0.6 ml DMEM with 10% FBS was added into the lower chamber. Chambers were incubated at 5% CO_2 and 37˚C for 6 h. Following removal of the medium, cells were fixed on the lower side of the insert filter by incubating with 4% paraformaldehyde (Sigma-Aldrich; Merck-Millipore) for 15 min, and cells that did not migrate on the upper side of the filter membrane were removed with a cotton swab. The cells on the lower side of the insert filter were stained with 0.1% Crystal Violet (Sigma-Aldrich; Merck-Millipore) for 10 min. The number of the cells on the lower side of the filter were then visualized and counted under a microscope (IX-70; Olympus Corporation, Tokyo, Japan) after washing with PBS (Gibco; Thermo Fisher Scientific, Inc.). The cells that had migrated through the membrane were stained and counted, and chondrocyte migration was expressed as the percentage of the total number of cells that had migrated.

Statistical analysis. Experiments were performed in triplicate and results are expressed as mean ± standard deviation. Statistical analyses were performed using the SPSS statistical software package (version, 17.0; SPSS, Inc., Chicago, IL, USA). Differences between control and treated groups were analyzed using non-parametric Mann-Whitney U tests. $P<0.01$ was considered to indicate a statistically significant difference.

Results

miR-195 targets GIT1. Multiple miRNAs may target the same gene, and an miRNA can also target multiple genes, for example, miR-425, miR-376a and miR-138 may target GIT1, and miR-195 can target ZNF367, HDGF and CHEK1,. The present study used common bioinformatic algorithms to predict miRNAs that target GIT1. Previous studies have demonstrated that miR-195 serves a pivotal role in osteogenesis and bone development (17-19), however, there has been less research into the effect of other miRNAs on bone development, thus, the present study selected miR-195 for further investigation. As shown in Fig. 1A and B, the GIT1 3'-UTR was observed to contain miR-195 binding sites. In the present study, dual-luciferase reporter assay was used to verify binding of miR-195 to GIT1. As shown in Fig. 1B, luciferase expression levels decreased significantly following transfection of cells with vectors containing GIT1 3'-UTR clones plus miR-195 mimics ($P<0.01$). Conversely, luciferase expression levels were not significantly altered when the binding site was mutated (Fig. 1B). This demonstrated that miR-195 may bind to a site in the GIT1 3'-UTR, which suggests that GIT1 may be a target of miR-195.

Ago2 is a core component of the RNA-induced silencing complex that associates with miRNAs and their mRNA targets (20). Therefore, immunopurification of Ago2 under

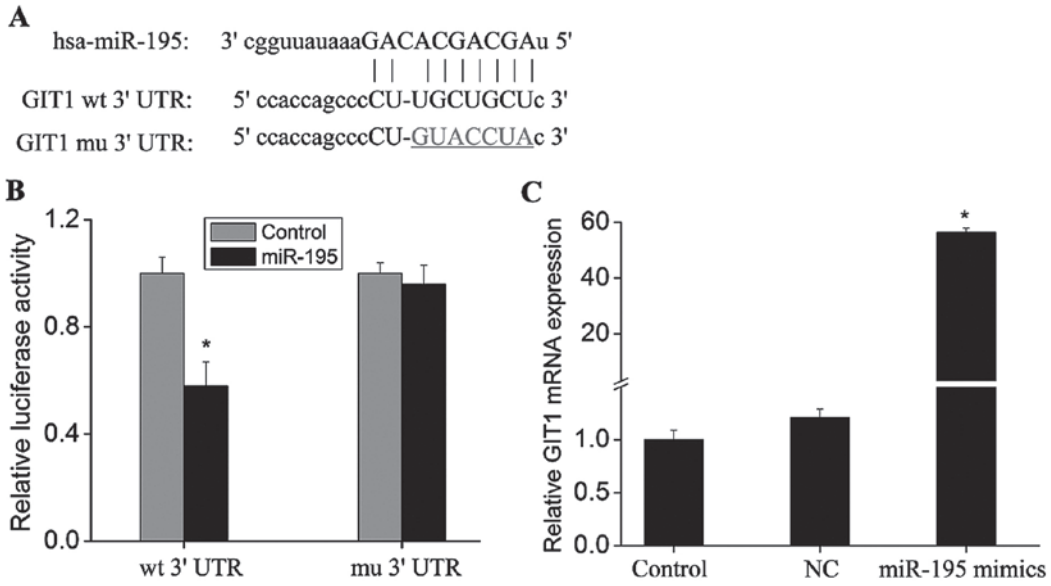

Figure 1. Prediction of miR-195 target site in GIT1 3'-UTR. (A) miR-195 target sites in the conservative sequence of the GIT1 3'-UTR were identified using common bioinformatic algorithms (miRanda, TargetScanHuman, miRBase and miRWalk), as indicated by capital letters. The putative miR-195 binding site was mutated (underlined nucleotides). (B) Luciferase expression in HEK293A cells transfected with vectors containing the wt GIT1 3'-UTR or mu GIT1 3'-UTR together with miR-195 mimics or controls. (C) The Ago2 monoclonal antibody was immobilized on Protein-G-Sepharose beads and incubated with HEK 293 cell lysates obtained following transfection with miR-195 mimics. After stringent washing, co-immunoprecipitated Ago-bound RNAs were subject to RT-qPCR to detect GIT1 mRNA expression. Data are presented as the mean ± standard deviation (n=3). *P<0.01 vs. the controls. miR-195, microRNA-195; GIT1, G-protein-coupled receptor kinase interacting protein-1; UTR, untranslated region; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; NC, negative control miRNA transfection group; wt, wild-type; mu, mutant.

the appropriate conditions may retain associated miRNAs and mRNAs, thereby allowing miRNA targets to be identified. A monoclonal antibody against Ago2 was immobilized on Protein-G-Sepharose beads and incubated with HEK 293 cell lysates. Following stringent washing, the co-immunoprecipitated Ago-bound RNAs were extracted and subject to RT-qPCR analysis in order to detect GIT1 mRNA expression. Following transfection of miR-195 mimics, GIT1 mRNA levels were significantly higher when compared with controls (Fig. 1C; P<0.01). This further demonstrated that miR-195 may target and regulate GIT1 expression.

miR-195 inhibits the expression of GIT1 in chondrocytes. As GIT1 may be a target gene of miR-195, it is formally possible that miR-195 may regulate the expression of GIT1 in chondrocytes. In the current study, miR-195 mimics and inhibitors were transfected into human chondrocytes, and miR-195 and GIT1 expression was measured. The results indicated that a significant increase in miR-195 expression was associated with a significant downregulation in GIT1 mRNA and protein expression levels when compared with controls (Fig. 2; P<0.01). By contrast, when miR-195 expression was suppressed, GIT1 mRNA and protein expression increased significantly when compared with controls (Fig. 2B-D; P<0.01). These results suggest that miR-195 may regulate GIT1 expression in chondrocytes.

miR-195 inhibits chondrocyte proliferation through targeted regulation of GIT1 expression. The results presented so far suggest that miR-195 targets and regulates the expression of GIT1 in chondrocytes. However, the role and association of this interaction with the biological behavior of chondrocytes requires further investigation. In the present study, a BrdU assay was performed in order to investigate the effect of miR-195 on chondrocyte proliferation. As shown in Fig. 3, chondrocyte proliferation increased significantly when miR-195 expression was suppressed with miR-195 inhibitors, as well as following overexpression of GIT1 compared with the control group (P<0.01). By contrast, transfection with miR-195 mimics or GIT1 siRNA demonstrated the opposite effect on chondrocyte proliferation compared with the control group (Fig. 3). These results demonstrate that chondrocyte proliferation may be inhibited by miR-195, but promoted by GIT1 expression. When miR-195 and GIT1 overexpression vectors were co-transfected, the inhibitory effect of miR-195 on chondrocyte proliferation was significantly attenuated (Fig. 3; P<0.01). However, upon co-transfection with miR-195 and GIT1 overexpression vectors containing the wild-type 3'-UTR sequence, miR-195-mediated inhibition of chondrocyte proliferation was unaffected (Fig. 3). These results demonstrate that miR-195 may be involved in mediating chondrocyte proliferation by regulating GIT1 expression.

miR-195 inhibits chondrocyte migration through targeted regulation of GIT1 expression. Previous studies have demonstrated that a key function of GIT1 is to promote cell migration (21,22). Therefore, due to the observed putative role of miR-195 in regulating GIT1, it is possible that miR-195 may suppress cell migration. As shown in Fig. 4, upon transfection of miR-195 mimics in chondrocytes, the cell migration capacity decreased significantly when compared with controls (P<0.01). A similar effect was observed following transfection of cells with GIT1 siRNA (Fig. 4; P<0.01). Following transfection of miR-195 inhibitors, the migration capacity of chondrocytes

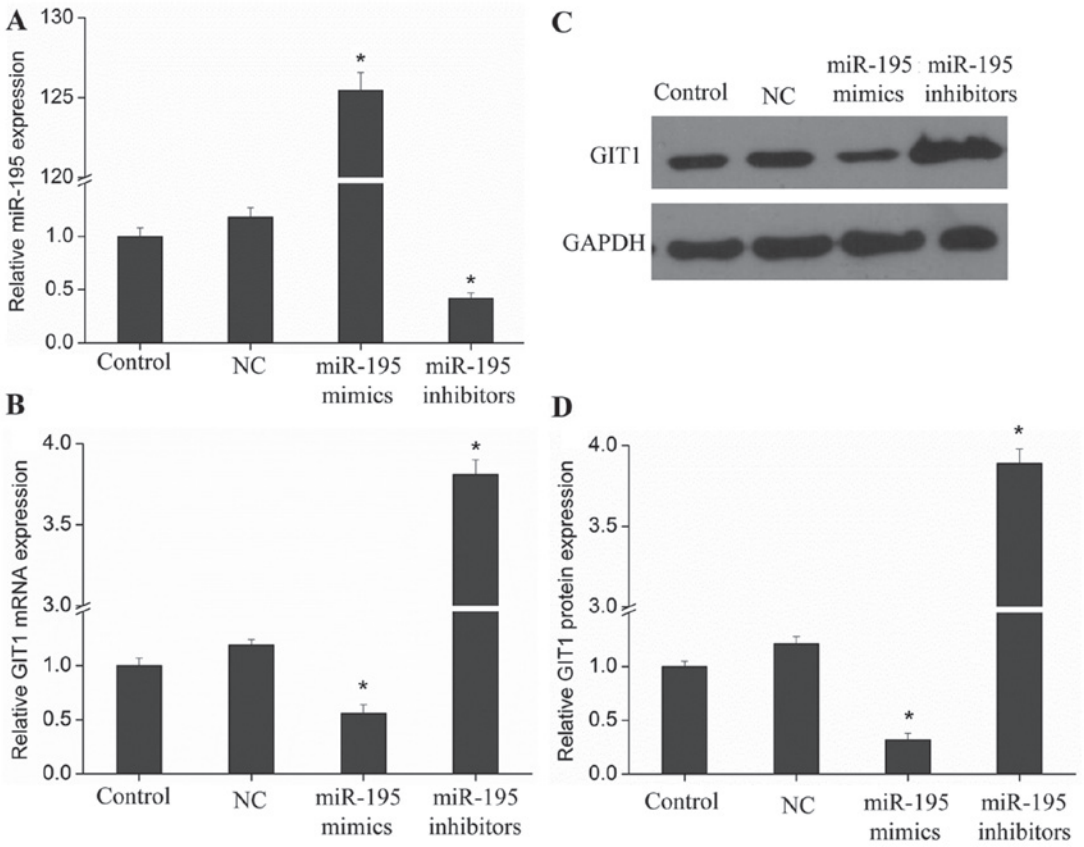

Figure 2. miR-195 inhibits the expression of GIT1 in chondrocytes. Human chondrocytes were transfected with miR-195 mimics or inhibitors. The mRNA expression levels of (A) miR-195 and (B) GIT1 were measured by RT-qPCR analysis. (C) Western blot analysis and (D) quantification of band intensities of GIT1 protein expression in chondrocytes following transfection with miR-195 mimics or inhibitors. GAPDH was used as a loading control. Experiments were performed at least in triplicate, and the results are expressed as the mean ± standard deviation. *P<0.01 vs. the control. miR-195, microRNA-195; GIT1, G-protein-coupled receptor kinase interacting protein-1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; NC, negative control.

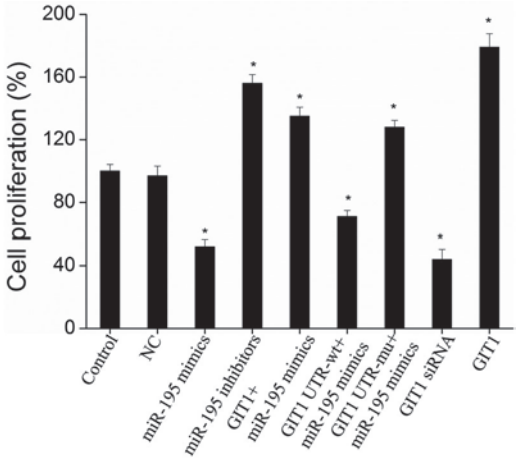

Figure 3. Role of miR-195 and GIT1 in chondrocyte proliferation. Chondrocytes were transfected with GIT1-expression vectors, miR-195 mimics, miR-195 inhibitors or GIT1 siRNA and cultured for 72 h. Cell proliferation was measured using a BrdU assay. The results are presented as the mean ± standard deviation, (n=3). *P<0.01 vs. the control. miR-195, microRNA-195; GIT1, G-protein-coupled receptor kinase interacting protein-1; NC, negative control; UTR, untranslated region; wt, wild-type; mut, mutant; siRNA, small-interfering RNA.

increased significantly compared with the control group (P<0.01). This was similar to the migration capacity observed following transfection of cells with GIT1 expression vectors (Fig. 4; P<0.01). Co-transfection of miR-195 mimics with GIT1 expression vectors attenuated the inhibitory effect of miR-195 on cell migration (Fig. 4). These results suggest that miR-195 may inhibit chondrocyte migration by regulating GIT1 expression.

Discussion

Chondrocytes are located in cartilage lacunae and possess supportive and protective roles in joint movement and weight-bearing. In addition, chondrocytes respond to wounds, stress and external stimuli to initiate cell repair and proliferation processes (1,2). The proliferation of chondrocytes is affected by various growth factors, cytokines and additional external conditions including, mechanical pressure and alterations in cell density (1,2).

An increasing number of studies have demonstrated that GIT1 demonstrates an important role in bone growth and development (3-6). For instance, Xiao *et al* (4) suggested that platelet-derived growth factor regulates chondrocyte proliferation through activation of the ERK1/2 signaling pathway via upregulation of GIT1 expression and Rac1 phosphorylation. In addition, an investigation into miRNA function was demonstrated to be involved in the differentiation and formation of human bone and joint tissues, including osteoblasts, osteoclasts

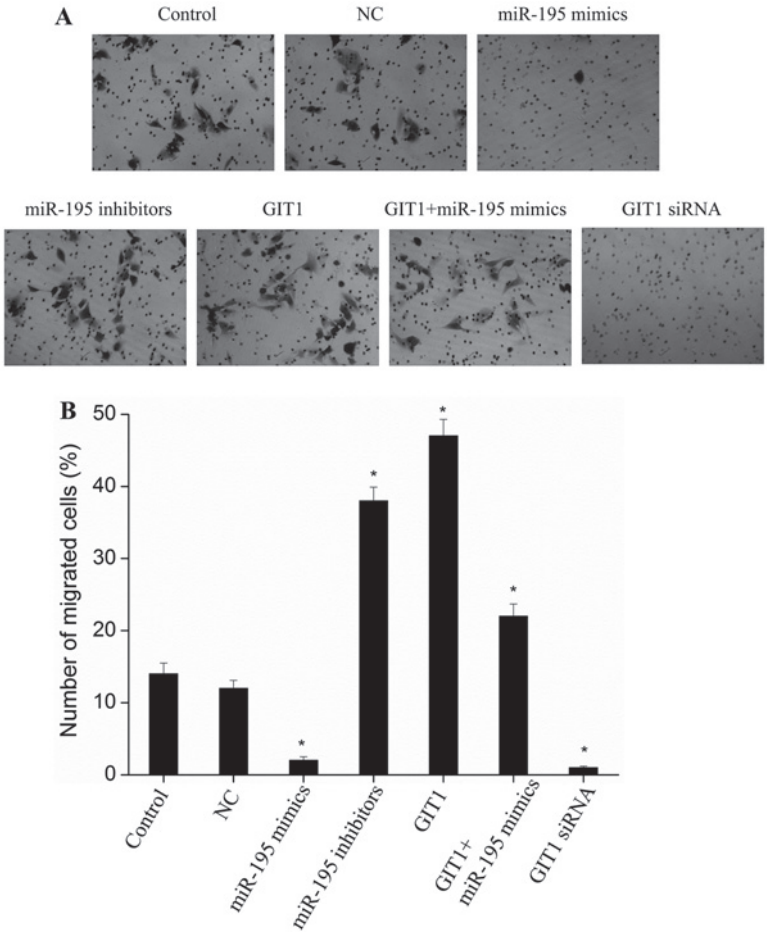

Figure 4. Role of miR-195 and GIT1 in chondrocyte migration. Cell migration capabilities were analyzed using a transwell assay. (A) Light microscope images of chondrocytes transfected with GIT1-overexpressing vectors, miR-195a-3p mimics, miR-195a-3p inhibitors or GIT1 siRNA, that were seeded onto matrigel-coated filters of cell culture inserts and incubated for 24 h. The images shown are representative of three independent experiments (magnification, x200). Cells that had migrated to the underside of the filters toward FBS-containing media were counted. (B) Quantitative analysis of the effects of miR-195 and/or GIT1 expression on chondrocyte migration capabilities. Data are presented as the mean ± standard deviation (n=3). *P<0.01 vs. the control. miR-195, microRNA-195; GIT1, G-protein-coupled receptor kinase interacting protein-1; FBS, fetal bovine serum; siRNA, small interfering RNA; NC, negative control.

and chondrocytes (12,13). Therefore, miRNAs may be an important focus of research concerning joint disease prevention and treatment. A previous study demonstrated that miR-1 regulates aggrecan expression in human chondrocytes, and is involved in regulating chondrocyte phenotypic stability (23). In addition, miR-1 serves an important regulatory role in the late differentiation of chondrocytes, and in maintaining the integrity of cartilage tissues (23).

The present study investigated the miRNAs that target GIT1 and demonstrated that miR-195 may target and regulate GIT1 due to the identification of a putative binding site in the GIT1 3'-UTR. To date, studies concerning miR-195 function in tumors have made significant progress (24-26). Zhou *et al* (24) indicated that miR-195 inhibited non-small cell lung cancer cell proliferation, migration and invasion by targeting the MYB proto-oncogene. However, studies investigating the role of miR-195 in bone growth and development are rare. According to the results of the current study, miR-195 demonstrated an inhibitory effect on the expression of GIT1 in chondrocytes, and may affect chondrocyte proliferation and migration by regulating GIT1.

According to the results of the current study, miR-195 inhibits chondrocyte proliferation and migration, and one pathway by which miR-195 may mediate this effect is through regulating GIT1. Consistent with these observations, the inhibitory effect of miR-195 on cell proliferation in additional cell types has been reported previously. Sekiya *et al* (27) demonstrated that downregulation of cyclin E1 expression by miR-195 accounted for the interferon-β-induced inhibition of hepatic stellate cell proliferation. In addition, Wang *et a* (28) demonstrated that miR-195 inhibited the proliferation and growth, and induced apoptosis of endometrial stromal cells by targeting the fractalkine gene. Grünhagen *et al* (17) identified the miR-497~195 cluster, a member of the miR-15 family, as being strongly upregulated during postnatal bone development *in vivo*, and late differentiation stages of primary osteoblasts cultured *in vitro*. Early expression of miR-195-5p was observed to inhibit osteoblast differentiation and mineralization. Using microarray and RT-qPCR analyses, miR-195-5p was observed to alter the gene regulatory network of osteoblast differentiation, and impair the induction of bone morphogenetic protein responsive genes. In addition, Bai *et al* (18) demonstrated that miR-195 significantly increased apoptosis and downregulated hypoxia-inducible factor 1-α mRNA expression simultaneously in hypoxic chondrocytes. According to the

results of the present study, miR-195 was observed to inhibit chondrocyte cell proliferation and migration, potentially through regulating GIT1 expression.

In conclusion, miR-195 may target and regulate the expression of GIT1 in chondrocytes. In addition, miR-195 inhibited the proliferation and migration of chondrocytes, likely through the targeted regulation of GIT1 expression. The results of the current study may provide a rationale for investigating the regulatory effects and underlying mechanisms of miRNAs in bone and chondrocyte tissues, and may provide a novel approach for understanding osteoarticular diseases.

Acknowledgements

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