lncRNA GAS5 regulates angiogenesis by targeting miR-10a-3p/VEGFA in osteoporosis

WEN WU, QIANG LI, YI-FENG LIU and YONG LI

Department of Spine Surgery, Brain Hospital of Hunan Province, Changsha, Hunan 410007, P.R. China

Received July 6, 2020; Accepted January 28, 2021

DOI: 10.3892/mmr.2021.12350

Abstract. Osteoporosis is a severe bone disease commonly occurring in older males and postmenopausal females. Previous studies have shown that long non-coding (lnc) RNA growth arrest-specific 5 (GAS5) serves an important role in osteoporosis. However, its role is unclear and requires further exploration. The relative expression levels of GAS5 and miR-10a-3p in the serum samples of patients with osteoporosis, as well as the relative expression levels of GAS5, microRNA (miR)-10a-3p and vascular endothelial growth factor A (VEGFA) mRNA in osteoblasts, were detected by reverse transcription-quantitative PCR. ELISA and western blotting were used to detect the expression levels of VEGFA. A Matrigel angiogenesis test was used to assess the effects on angiogenesis. RNA binding interactions between GAS5/miR-10a-3p and miR-10a-3p/VEGFA were evaluated using dual-luciferase reporter assays. Furthermore, the effects of the GAS5/miR-10a-3p/VEGFA axis were investigated via ELISA, western blotting and Matrigel angiogenesis. GAS5 was significantly downregulated and miR-10a-3p was upregulated in patients with osteoporosis. Overexpression of GAS5 promoted angiogenesis. GAS5 acted as a sponge of miR-10a-3p; VEGFA was a target gene of miR-10a-3p. GAS5 induced angiogenesis by inhibiting miR-10a-3p and enhancing VEGFA expression. These results indicated that GAS5 overexpression increased angiogenesis by inhibiting miR-10a-3p, promoting the expression of VEGFA. The present study revealed a novel mechanism and provided novel targets for the clinical treatment of osteoporosis.

Correspondence to: Dr Yong Li, Department of Spine Surgery, Brain Hospital of Hunan Province, 427 Furong Middle Road (Section 3), Changsha, Hunan 410007, P.R. China
E-mail: 340377324@qq.com

Abbreviations: lncRNA, long non-coding RNA; GAS5, growth arrest-specific 5; VEGFA, vascular endothelial growth factor A; PMOP, postmenopausal osteoporosis; miRNA/miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR, CM, conditioned medium

Key words: lncRNA GAS5, microRNA-10a-3p, angiogenesis, osteoporosis, VEGFA

Introduction

Osteoporosis is a metabolic bone disease characterized by reduced bone tissue volume per unit volume and degeneration of the bone tissue microstructure, resulting in decreased bone strength, increased brittleness and the risk of fracture (1). The morbidity of osteoporosis increases with age in both males and postmenopausal females (2), mainly due to advanced age and estrogen deficiency (3), affecting >50% of females >50 years of age (4). Other pathological factors are associated with immune responses, including chronic inflammation (5) or immune regulation (6), as well as an imbalance in osteoclast or osteoblast differentiation (7). Various efforts have been taken to improve the diagnosis and treatment of osteoporosis; however, high recurrence rates, high cost of treatment, poor patient compliance, poisonous side effects of treatment drugs and malabsorption render the clinical efficacy suboptimal (8). Therefore, there are a number of challenges associated with the prevention and treatment of osteoporosis. Accordingly, exploring the molecular mechanisms underlying osteoporosis is necessary and meaningful to identify a new target and treatment direction.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs without protein-coding function (9). They are typically 200-100,000 nucleotides in length and produced by RNA polymerase II (10). However, lncRNAs are involved in multiple cellular processes and serve various roles in epigenetic, transcriptional and post-transcriptional regulation of gene expression (10-15). Certain studies have reported that lncRNAs play important regulatory roles in the occurrence and development of osteoporosis. For example, Zhang *et al* (16) demonstrated that lncRNA MSC-antisense (AS)1 promotes the osteogenic differentiation of bone marrow-derived stromal cells (BMSCs) and alleviates the progression of osteoporosis by sponging miR-140-5p to upregulate bone morphogenic protein (BMP)2. Conversely, Wang *et al* (17) reported that lncRNA maternally expressed 3 (MEG3) inhibits the osteogenic differentiation of bone marrow mesenchymal stem cells from postmenopausal osteoporosis (PMOP) by targeting the expression of microRNA (miRNA/miR)-133a-3p. Ma *et al* (18) showed that lncRNA neighboring enhancer of FOXA2 is downregulated in PMOP and associated with the course of treatment and recurrence, which may be involved in the inhibition of IL-6 secretion. lncRNA DANCR is upregulated in blood mononuclear cells and promotes bone resorption

by releasing TNF-α and IL-6, resulting in osteoporosis (19). Downregulation of lncRNA ANCR promotes osteoblast differentiation by targeting enhancer of zeste homolog 2 and regulating runt-related transcription factor 2 (RUNX2) expression (20,21). These observations suggest that lncRNAs are closely associated with the development of osteoporosis. lncRNA growth arrest-specific 5 (GAS5) isolated at the lymphoma-associated chromosomal locus (1q25) and exerts an important regulatory role in tumorigenesis (22). Additionally, GAS5 negatively regulates cell survival, participates in the development of bone diseases and is upregulated in patients with osteoarthritis (23). However, Feng *et al* (24) reported that GAS5 is downregulated in patients with osteoporosis, and GAS5 overexpression promotes the osteogenic differentiation of human mesenchymal stem cells by regulating miR-498 to upregulate RUNX2 expression, which alleviates the development of osteoporosis. Thus, the mechanisms of GAS5 in osteoporosis require further exploration.

miRNAs are functional non-coding RNAs that can recognize specific target genes by incomplete base pairing, inhibiting the translation of these target genes (25). As potential therapeutic targets or biomarkers, certain miRNAs have gained increasing attention in osteoporosis, including miR-144-3p (26) and miR-132-3p (27). However, only one study has reported the role of miR-10a-3p in osteoporosis. Kaempferol promotes BMSC osteogenic differentiation and improves osteoporosis by downregulating miR-10a-3p (28), a topic that requires further exploration. Vascular endothelial growth factor A (VEGFA), originally identified as an endothelial-specific mitogen and permeability factor, serves a critical role in promoting angiogenesis, which is essential and dependent on bone formation (29,30). Additionally, VEGFA is expressed during osteoblast differentiation, and the exogenous addition of VEGFA can stimulate osteoblast-like cell differentiation, which can attenuate osteoporosis (31). Angiogenesis plays a positive regulatory role in attenuating osteoporosis by enhancing bone formation (29). However, the association between miR-10a-3p and VEGFA remains unclear, and the effects of miR-10a-3p on VEGFA regulation in angiogenesis warrant further exploration.

The present study investigated the effect of knockdown or overexpression GAS5 or miR-10a-3p on angiogenesis of osteoblasts. The present study aimed to verify whether GAS5 overexpression regulates angiogenesis via miR-10a-3p/VEGFA, which may provide novel targets and pathways for the clinical treatment of osteoporosis.

Materials and methods

Clinical sample collection. Blood samples were obtained (March 2019 to June 2019; Brain Hospital of Hunan Province, Changsha, China) from median cubital vein of patients with osteoporosis (n=10; 6 females and 4 males; age, 56-73 years) and healthy subjects (n=10; 5 females and 5 males; age, 57-72 years). The inclusion criteria were as follows: Patients who were diagnosed with osteoporosis via X-ray examination and without other diseases; and healthy subjects who exhibited normal bone density via X-ray examination and without other diseases. The basic information of the patients and healthy controls is presented in Table I. Patients with rheumatoid arthritis and other metabolic diseases were excluded. The healthy subjects had no bone diseases and could walk freely. The blood samples were centrifuged (1,000 x g, 5 min at room temperature), serum was collected and was frozen and stored at -80˚C. The study was approved by the Brain Hospital of Hunan Province (approval no. 2019058). Informed consent was obtained from all the participants before sample collection.

Detection of bone mineral density (BMD). The BMD of the patients with osteoporosis and healthy subjects was determined via peripheral dual-energy X-ray absorptiometry (PIXImus II; Lunar; GE Healthcare). They were arranged in the prone position, and an image was acquired in 5 min. The BMD (g/cm^2) of the entire body was determined except the head.

Cell culture. Human osteoblasts (hFOB1.19) and human umbilical vein endothelial cells (HUVECs) were cultured in DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Invitrogen; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.5 μg/ml fungizone at 37˚C in a humidified 5% CO_2 incubator.

Cell transfection. pcDNA-GAS5 or pcDNA-VEGFA (pcDNA; control), short hairpin RNA (sh)GAS5 (shNC; control) were synthesized by GeneCopoeia, Inc. miR-10a-3p mimics (5'-CAAAUUCGGAUCUACAGGGUAUU-3'), miR-10a-3p inhibitor (5'-CACAAAUUCGGAUCUACAGGGUA-3'), NC (mimics NC, 5'-UUCUCCGAACGUGUCACGUTT-3'); inhibitor NC, (5'-CAGUACUUUUGUGUAGUACAA-3') were purchased from Sangon Biotech Co., Ltd. Briefly, 2Then, $1x10^4$ hFOB1.19 cells were transfected with pcDNA-GAS5 or pcDNA-VEGFA (0.5 μg), miR-10a-3p mimics/inhibitor (50 nM) or mimics NC/inhibitor NC (50 nM) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h, during which time the medium was not replaced. The expression of the relative genes in transfected cells was subsequently determined via reverse transcription-quantitative (RT-q)PCR.

RT-qPCR. Total RNA in the cells was extracted using TRIzol® reagent (Takara Bio, Inc.). The RNA concentration and purity were detected via spectrophotometry. Subsequently, 1 μg total RNA was reverse transcribed into cDNA using a PrimeScript™ RT Reagent kit with gDNA Eraser (Takara Bio, Inc.) according to the manufacturer's protocol. qPCR was performed according to the manufacturer's instructions of an SYBR Premix Ex Taq II kit (Takara Bio, Inc.) using an ABI Prism 7500 HT Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction procedures were as follows: Pre-denaturation at 94˚C for 10 min, followed by 40 cycles of denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension for 60 sec at 72˚C. The relative expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method (32) and were normalized to GAPDH or U6. The primer sequences were as follows: GAS5 forward, 5'-CTTGCCTGGACCAGCTTAAT-3' and reverse, 5'-CAAGCCGACTCTCCATACCT-3'; miR-10a-3p forward, 5'-GCGCGCAAATTCGTATCTAGG-3' and reverse, 5'-GTCGTATCCAGTGCAGGGTCC-3'; VEGFA forward, 5'-CCCGGGCCTCGGTTCCAG-3' and reverse, 5'-GTCGTGGGTGCAGCCTGGG-3';

Table I. Basic information of patients with osteoporosis and healthy individuals in the present study.

A, Patients with osteoporosis

Patient number	Sex	Age, years
1	Female	62
2	Female	56
3	Male	71
4	Female	64
5	Male	70
6	Female	66
7	Female	58
8	Male	67
9	Female	60
10	Male	73

B, Healthy individuals

Patient number	Sex	Age, years
1	Female	59
2	Male	64
3	Male	69
4	Female	63
5	Female	57
6	Male	68
7	Male	62
8	Female	60
9	Male	72
10	Female	65

GAPDH forward, 5'-GAGTCAACGGATTTGGTCGTT-3' and reverse, 5'-TTGATTTTGGAGGGATCTCG-3'; and U6 forward, 5'-TGCGGGTGCTCGCTTCGGCAGC-3' and reverse, 5'-CCAGTGCAGGGTCCGAGGT-3'.

ELISA. The cells were treated with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime Institute of Biotechnology). VEGFA was examined using a Human VEGF ELISA kit (Abcam; cat. no. ab222510) according to the manufacturer's protocols.

Western blot analysis. Total proteins in cells were extracted using RIPA lysis buffer containing protease and phosphatase inhibitors (Selleck Chemicals). Protein concentrations were determined using a BCA kit. The protein samples (25 μg/lane) were separated via 12% SDS-PAGE and transferred onto PVDF membranes (EMD Millipore). The membranes were blocked in 5% BSA (Beijing Solarbio Science & Technology Co., Ltd.; cat. no. SW3015) for 1 h at room temperature and incubated with anti-VEGFA (Abcam; 1:1,000; cat. no. ab1316) and anti-GAPDH (Abcam; 1:1,000; cat. no. ab8245) antibodies at 4˚C overnight. On the following day, the membranes were incubated with HRP-conjugated secondary antibodies diluted at 1:3,000 (Abcam; cat. no. ab97040) at room temperature for 2 h. The protein bands were visualized using an Immobilon Western Chemilum HRP Substrate (EMD Millipore, WBKLS0100) and analyzed using ImageJ software 1.52a (National Institutes of Health).

Matrigel angiogenesis. Conditioned medium (CM) was collected from hFOB1.19 cells transfected with GAS5 or miR-10a-3p. Matrigel was slowly melted at 4˚C overnight, and 10 μl was added to each well of the angiogenic microslide. After Matrigel solidification, $2x10^5$ cells/ml of a HUVEC cell suspension was prepared. Next, 50 μl cell suspension (~10,000 cells) was added to the Matrigel and then blocked with a coverslip. After incubating with 100 μl/well of the indicated CM for 8 h at 37˚C, tubular structures were captured in five fields of view using a light inverted microscope (cat. no. IX73; Olympus Corporation) at x100 magnification and analyzed using ImageJ software 1.52a (National Institutes of Health).

Dual-luciferase reporter assay. The interaction between GAS5 and miR-10a-3p, and VEGFA and miR-10a-3p was predicted by StarBase V2.0 (starbase.sysu.edu.cn). To determine the relationship between GAS5 and miR-10a-3p, as well as between miR-10a-3p and VEGFA, wild-type (WT) or mutant (MUT) sequences of the 3'-untranslated region of GAS5 or VEGFA were inserted into pmirGLO luciferase vectors to generate GAS5-WT, GAS5-MUT, VEGFA-WT and VEGFA-MUT vectors (Promega Corporation). Osteoblasts were co-transfected with the above plasmids and miR-10a-3p or mimics NC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). The transfected cells were washed with PBS, and the luciferase activity was measured after 48 h using a dual-luciferase assay system (Promega Corporation). The firefly luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. For statistical analyses, one-way ANOVA (multiple comparisons) and Student's t-test (two comparisons) were performed using SPSS software (v12.0; SPSS, Inc.). Tukey's post hoc test was used for multiple comparisons. Correlation was assessed by Pearson's coefficient. The results were presented as the mean ± SD of three independent experiments. $P<0.05$ was considered to indicate a statistically significant difference.

Results

lncRNA GAS5 is downregulated and miR-10a-3p is upregulated in patients with osteoporosis. The bone mineral density of patients with osteoporosis and healthy subjects was detected, revealing that patients exhibited significantly reduced bone mineral density compared with healthy subjects (Fig. 1A). Representative X-ray images of patients with osteoporosis and healthy subjects are shown in Fig. S1. Serum was collected from the blood samples of patients with osteoporosis and healthy subjects to detect the levels of GAS5 and miR-10a-3p via RT-qPCR. The expression of GAS5 was significantly downregulated in patients compared with the healthy subjects (Fig. 1B). Conversely, the levels of miR-10a-3p were significantly increased in patients (Fig. 1C). Additionally,

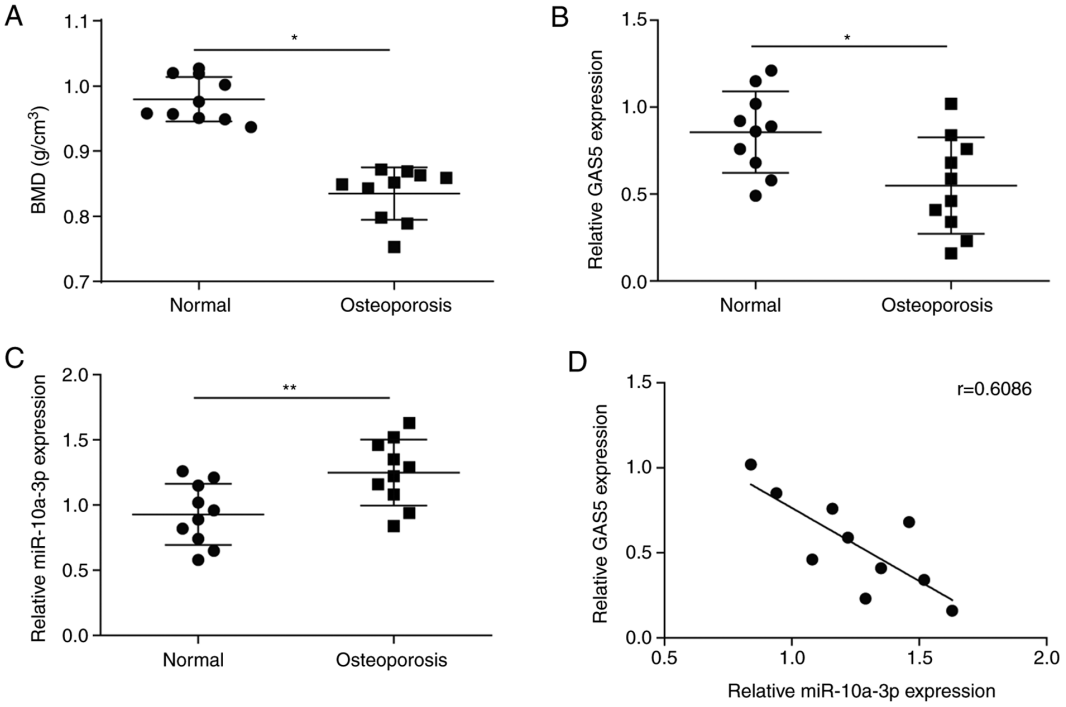

Figure 1. Long non-coding RNA GAS5 is downregulated and miR-10a-3p is upregulated in patients with osteoporosis. (A) BMD of patients with osteoporosis and healthy subjects was detected (n=10/group). Levels of (B) GAS5 and (C) miR-10a-3p were detected via reverse transcription-quantitative PCR in the serum of patients with osteoporosis and healthy subjects. (D) Linear correlation between GAS5 and miR-10a-3p. *P<0.05, **P<0.01. GAS5, growth arrest-specific 5; miR, microRNA; BMD, bone mineral density.

there was a negative linear correlation between the expression of GAS5 and miR-10a-3p (Fig. 1D). These results indicated that GAS5 was downregulated and miR-10a-3p was upregulated in osteoporosis.

Overexpression of lncRNA GAS5 effectively promotes angiogenesis. GAS5 was overexpressed in osteoblasts to investigate the effects of GAS5. RT-qPCR analysis that GAS5 expression is significantly increased in osteoblasts after GAS5 transfection compared with in the untransfected and empty vector control groups (Fig. 2A), indicating that GAS5 was successfully transfected in osteoblasts. The levels of VEGFA were detected via ELISA and western blotting. The levels of VEGFA were significantly elevated in osteoblasts after GAS5 transfection (Fig. 2B and C), indicating that GAS5 induced the upregulation of VEGFA. Additionally, a Matrigel angiogenesis assay revealed increased angiogenesis for HUVECs treated with CM from the GAS5 group compared with the control and empty vector groups (Fig. 2D), implying that GAS5 induced angiogenesis via VEGFA. Thus, GAS5 overexpression promoted angiogenesis by increasing the levels of VEGFA.

lncRNA GAS5 acts as a sponge of miR-10a-3p. A negative correlation was observed between GAS5 and miR-10a-3p, but the upstream or downstream relationship remains unknown. Therefore, the binding site between miR-10a-3p and GAS5 was predicted using StarBase V2.0 (Fig. 3A). A dual-luciferase reporter assay showed that the luciferase activity in the GAS5-WT group was significantly decreased following co-transfection with miR-10a-3p compared with mimics NC, indicating that GAS5 interacted with miR-10a-3p. When GAS5 was mutated, luciferase activity was not notably affected by transfection with miR-10a-3p compared with mimics NC (Fig. 3B). These results indicated an association between GAS5 and miR-10a-3p.

The expression levels of GAS5 and miR-10a-3p were detected in osteoblasts following transfection with GAS5 or shGAS5. The expression of GAS5 was upregulated in osteoblasts after transfection with GAS5 (Fig. 3C), while the expression of miR-10a-3p was downregulated (Fig. 3D). Opposing effects were observed after transfection with shGAS5 (Fig. 3C and D). These results indicated that GAS5 targeted miR-10a-3p.

VEGFA is a target gene of miR-10a-3p. The above findings indicated that GAS5 induced downregulation of miR-10a-3p. However, whether a targeting relationship exists between miR-10a-3p and VEGFA remains unknown. Therefore, a binding site between miR-10a-3p and VEGFA was predicted by StarBase V2.0 (Fig. 4A). A dual-luciferase reporter assay showed that miR-10a-3p bound to VEGFA in a targeted manner (Fig. 4B). After transfection with miR-10a-3p mimics, miR-10a-3p was upregulated and VEGFA mRNA was downregulated. When the miR-10a-3p inhibitor was transfected, opposing effects were observed (Fig. 4C and D). Similar findings were observed at the protein level (Fig. 4E). These results suggested that miR-10a-3p inhibited the expression of its target VEGFA.

lncRNA GAS5 promotes angiogenesis via the miR-10a-3p/VEGFA axis. Next, the mechanism underlying the promotion of angiogenesis by GAS5 was explored.

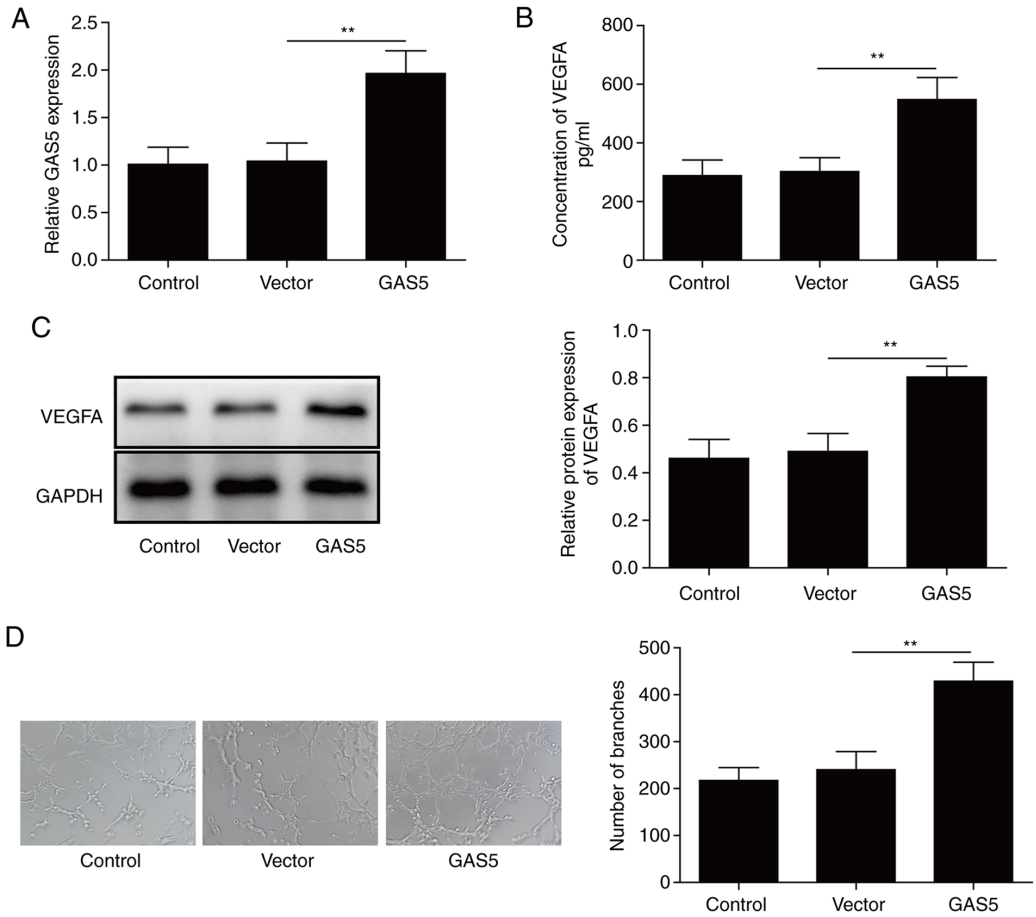

Figure 2. Overexpression of long non-coding RNA GAS5 promotes angiogenesis. (A) Expression of GAS5 was detected via reverse transcription-quantitative PCR after GAS5 transfection. (B) VEGFA levels were measured via ELISA. (C) VEGFA levels were assessed via western blotting. (D) Angiogenesis of human umbilical vein endothelial cells exposed to conditioned medium from different transfection groups was assessed using a Matrigel angiogenesis assay. **P<0.01. GAS5, growth arrest-specific 5; miR, microRNA; VEGFA, vascular endothelial growth factor A.

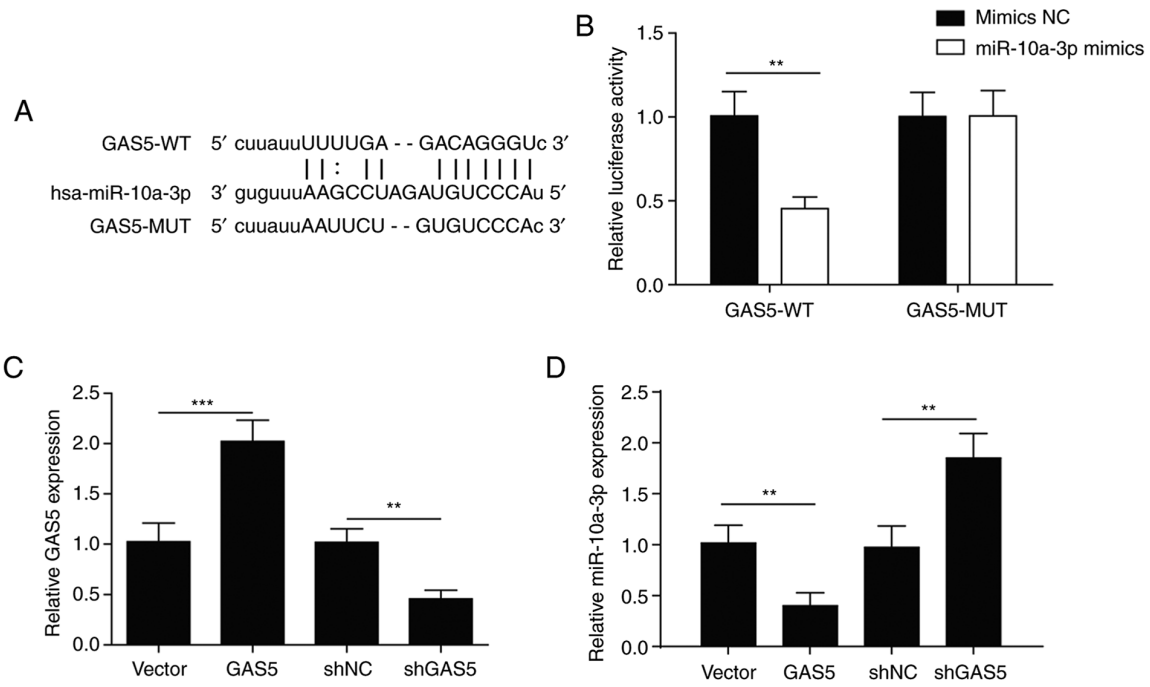

Figure 3. Long non-coding RNA GAS5 acts as a sponge of miR-10a-3p. (A) Binding site between GAS5 and miR-10a-3p was predicted by StarBase V2.0. (B) Binding relationship between GAS5 and miR-10a-3p was evaluated using a dual-luciferase reporter assay. (C) Expression of GAS5 was measured via RT-qPCR after GAS5 transfection. (D) RT-qPCR was used to assess the expression of miR-10a-3p after GAS5 transfection. **P<0.01, ***P<0.001. GAS5, growth arrest-specific 5; miR, microRNA; NC, negative control; WT, wild-type; MUT, mutant; sh, short hairpin RNA; RT-qPCR, reverse transcription-quantitative PCR.

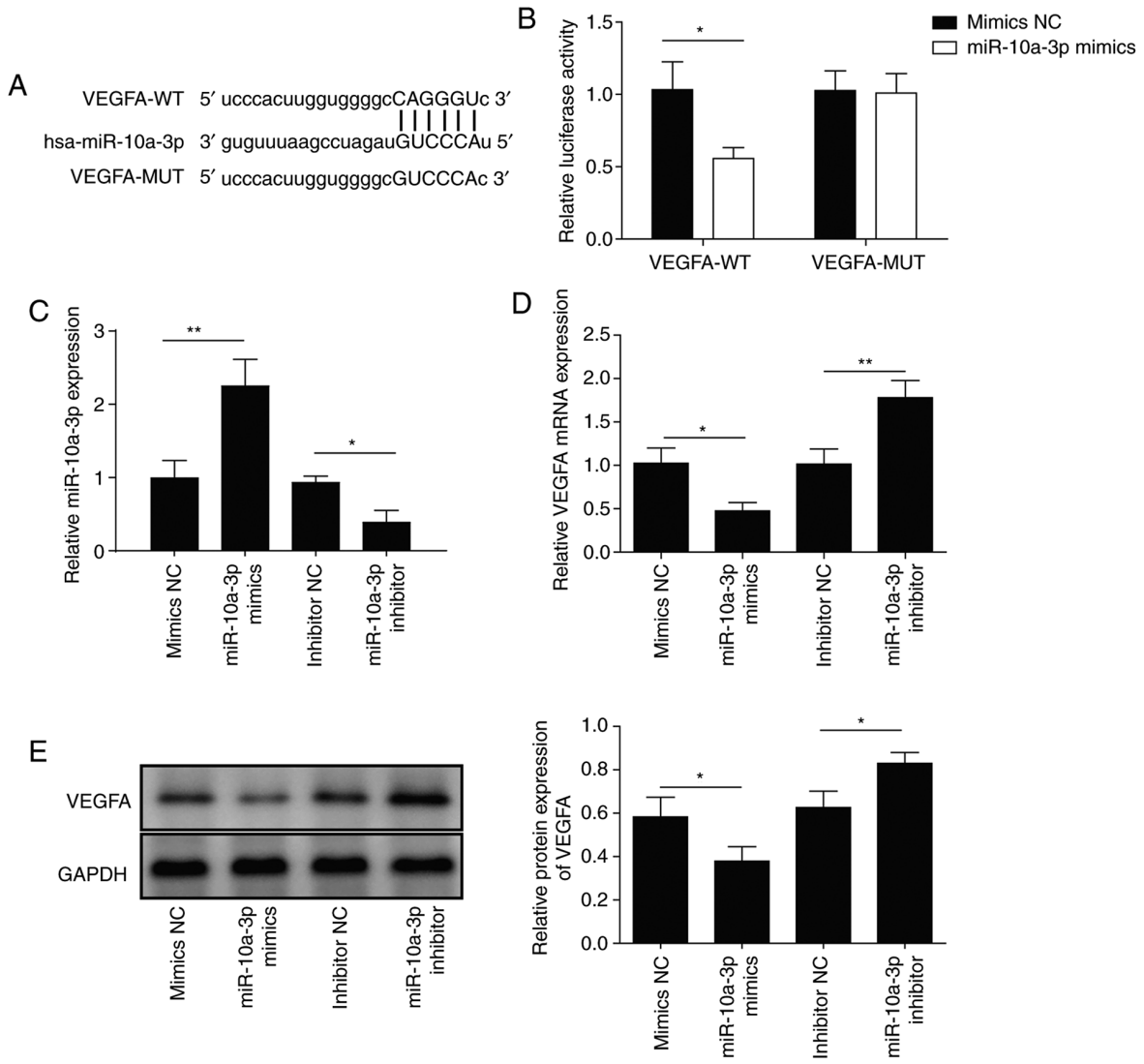

Figure 4. VEGFA is a target gene of miR-10a-3p. (A) Bioinformatics analysis predicted a binding site between miR-10a-3p and VEGFA. (B) Dual-luciferase reporter assay was performed to analyze the relationship between miR-10a-3p and VEGFA in osteoblasts. (C) Expression of miR-10a-3p in osteoblasts was assessed via RT-qPCR. (D) RT-qPCR was used to measure the mRNA expression levels of VEGFA in osteoblasts. (E) Protein expression of VEGFA in osteoblasts was assessed via western blotting. *P<0.05, **P<0.01. miR, microRNA; VEGFA, vascular endothelial growth factor A; NC, negative control; WT, wild-type; MUT, mutant; RT-qPCR, reverse transcription-quantitative PCR.

After transfection with GAS5 or VEGFA overexpression vectors (Fig. S2), the expression of GAS5 was significantly increased, while miR-10a-3p was significantly downregulated (Fig. 5A and B), and the mRNA and protein levels of VEGFA were increased (Fig. 5C-E). After transfection with miR-10a-3p mimics, miR-10a-3p was significantly increased (Fig. 5B), and the mRNA and protein levels of VEGFA were decreased (Fig. 5C-E). When GAS5 and miR-10a-3p mimics were transfected simultaneously, their effects were offset; however, the addition of VEGFA attenuated the inhibition of VEGFA expression by miR-10a-3p mimics (Fig. 5C-E). These results demonstrated that GAS5 regulated the expression of VEGFA by inhibiting miR-10a-3p.

Moreover, GAS5 overexpression promoted angiogenesis, whereas miR-10a-3p mimics exhibited an inhibitory effect. The presence of miR-10a-3p suppressed angiogenesis after GAS5 overexpression. However, the overexpression of VEGFA reversed the inhibition by miR-10a-3p mimics of angiogenesis (Fig. 5F), indicating that GAS5 promoted angiogenesis by increasing the expression of VEGFA.

In summary, GAS5 was downregulated in patients with osteoporosis, which induced upregulation of its target miR-10a-3p. Subsequently, miR-10a-3p inhibited the expression of its target VEGFA to suppress angiogenesis. Thus, the present study indicated that GAS5 promoted angiogenesis via the miR-10a-3p/VEGFA axis in osteoporosis.

Discussion

Osteoporosis is a severe bone disease, resulting in decreased bone strength, and increased fragility and risk of fracture, resulting in substantial harm to postmenopausal women and older men (33). Anti-resorptive agents (such as bisphosphonates and selective estrogen receptor modulators) and anabolic drugs that stimulate bone formation (including parathyroid hormone analogues and sclerostin inhibitors)

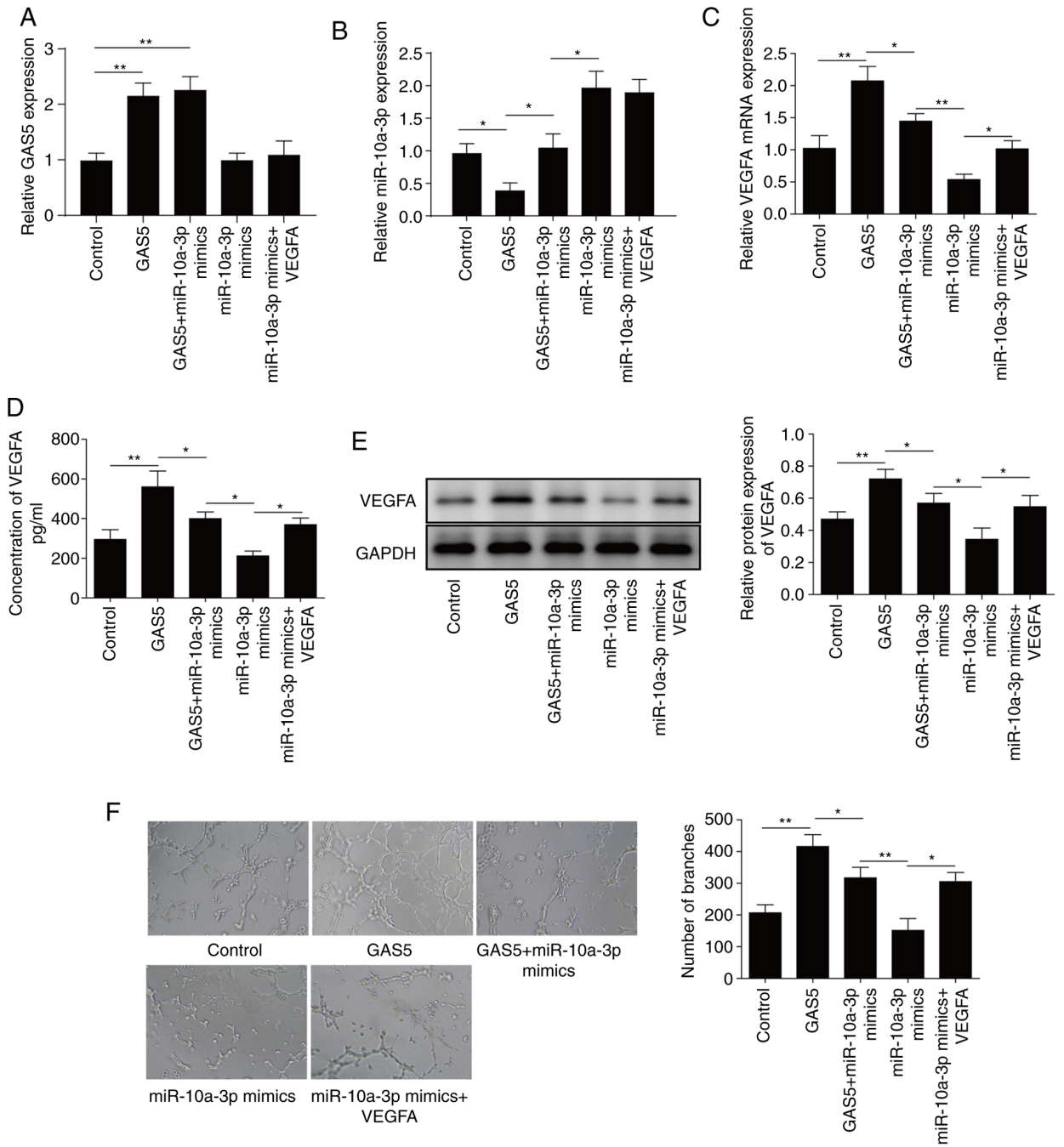

Figure 5. Long non-coding RNA GAS5 promotes angiogenesis via the miR-10a-3p/VEGFA axis. RT-qPCR was performed to detect the expression levels of (A) GAS5 and (B) miR-10a-3p in osteoblasts. (C) mRNA expression of VEGFA in osteoblasts was measured via RT-qPCR. (D) Levels of VEGFA were analyzed via ELISA. (E) Western blotting was performed to evaluate the protein levels of VEGFA in osteoblasts. (F) Angiogenesis was assessed using a Matrigel-based angiogenesis assay. *P<0.05, **P<0.01. GAS5, growth arrest-specific 5; miR, microRNA; VEGFA, vascular endothelial growth factor A; RT-qPCR, reverse transcription-quantitative PCR.

are current treatment strategies for osteoporosis (34). Previously, no study has compared the anti-fracture efficacy of different bisphosphonates, but recent evidence suggests that zoledronate treatment is more effective than risedronate or alendronate (35). Alendronate and calcium have been reported to exhibit higher efficacy when combined with oral Chinese herbal medicines in the treatment of senile osteoporosis (36). Despite their efficacy, long-term adherence remains a challenge due to the severe side effects and loss of potency (34).

A number of studies have investigated osteoporosis at the genetic level, and it has been reported that lncRNAs serve important roles in influencing osteoporosis. Examples of lncRNAs that promote osteogenic differentiation and alleviate the progression of osteoporosis include lncRNA MSC-AS1 (16) and GAS5 (23,24). Conversely, lncRNA MEG3 (17), lncRNA DANCR (19) and lncRNA-ANCR (20,21) inhibit osteogenic differentiation, resulting in osteoporosis. lncRNA GAS5 is reported to alleviate the development of osteoporosis via the miR-498/RUNX2 (24) and miR-135a-5p/FOXO1

pathways (37). However, treatments for osteoporosis using lncRNA GAS5-targeted drugs have not been reported. In the present study, it was demonstrated that GAS5 overexpression downregulated its target miR-10a-3p, which subsequently induced the upregulation of VEGFA and promoted angiogenesis, indicating potential for the treatment of osteoporosis. The study described a novel mechanism and novel targets with relevance for the treatment of osteoporosis.

miRNAs decrease the expression of their targets via specific binding, thus serving regulatory roles in cells. miR-133a promotes bone loss by altering the serum levels of osteoclastogenesis-related factors, decreasing lumbar spine BMD and altering bone histomorphology (38). miR-208a-3p, miR-155-5p and miR-637 were significantly upregulated in postmenopausal and premenopausal patients with osteoporosis, suggesting their association with disease pathogenesis (39). miR-19b-3p promotes the proliferation and osteogenic differentiation of BMSCs, revealing a role for miR-19b-3p in postmenopausal osteoporosis (40). However, no study has reported on the pro-angiogenic role of miR-10a-3p in osteoporosis. In the present study, the downregulation of miR-10a-3p by GAS5 overexpression increased the levels of VEGFA. These data are the first to indicate potential roles for GAS5 and miR-10a-3p in osteoporosis.

Several signaling pathways have been reported to be involved in the occurrence and development of osteoporosis, such as the JNK pathway (41), Wnt/β-catenin pathway (42), Janus kinase 2/STAT3 signaling pathway (43) and BMP6/Smad1/5/9 pathway (44), through which various proteins are targeted to regulate cell proliferation and osteogenic differentiation. VEGFA can stimulate the differentiation of osteoblast-like cells (27) and influence BMD in osteoporosis (45). In the present study, VEGFA was upregulated via the inhibition of miR-10a-3p by GAS5 overexpression; subsequently, VEGFA promoted osteoblastic angiogenesis. The present study further elucidated the role of VEGFA in osteoporosis and described a novel miR-10a-3p/VEGFA signaling pathway, providing new leads for understanding the mechanisms underlying the occurrence and development of osteoporosis.

The etiology of osteoporosis is complex, involving endocrine, immune, lifestyle, environmental and nutritional factors (46). Numerous studies have focused on the genetic level and have described several molecular signaling pathways. Therefore, investigating these molecular mechanisms may provide improved understanding of osteoporosis pathogenesis and potential therapeutic interventions. It was found that GAS5 overexpression upregulated the level of VEGFA by inhibiting miR-10a-3p, thus promoting angiogenesis. The present study is the first to describe the targeted relationship between GAS5, miR-10a-3p and VEGFA, increasing knowledge concerning this signaling pathway and providing a possible therapeutic target for osteoporosis.

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

WW conceived and designed the study. WW and YFL performed experiments and acquired data. WW and YL analyzed the data. QL assisted in data acquisition and interpretation and revised the manuscript. WW and YL prepared the manuscript. WW and YL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Brain Hospital of Hunan Province (approval no. 2019058). Informed consent was obtained from all participants prior to sample collection.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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