lncRNA-SNHG7-003 inhibits the proliferation, migration and invasion of vascular smooth muscle cells by targeting the miR-1306-5p/SIRT7 signaling pathway

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Abstract. Long non-coding RNAs (lncRNAs) have been discovered to participate in the progression of various types of disease and may be a promising biomarker for atherosclerosis (AS). The present study aimed to investigate the regulatory mechanisms of the lncRNA, small nucleolar RNA host gene 7-003 (SNHG7-003), on the proliferation, migration and invasion of vascular smooth muscle cells (VSMCs). VSMCs were first stimulated with oxidized low-density lipoprotein (ox-LDL) to simulate AS in a high fat environment. The expression levels of SNHG7-003, microRNA (miRNA/miR)-1306-5p and sirtuin 7 (SIRT7) were analyzed by reverse transcription-quantitative PCR and the effects of each of these factors on VSMC proliferation, migration and invasion were determined by Cell Counting Kit-8, wound healing and Transwell assays, respectively. Western blot analysis was also used to analyze the protein expression levels of α-smooth muscle actin (α-SMA), matrix metalloproteinase (MMP)2 and MMP9. The interactions between SNHG7-003 or SIRT7 and miR-1306-5p were determined using dual-luciferase reporter assays. The results revealed that the SNHG7-003 expression levels were downregulated in VSMCs exposed to ox-LDL, while the overexpression (OE) of SNHG7-003 significantly inhibited the proliferation, migration and invasion of VSMCs induced by ox-LDL. Transfection with miR-1306-5p mimic abrogated the effects of the inhibitory effects induced by SNHG7-003 OE. SIRT7 was validated to be a target gene of miR-1306-5p, exhibiting similar inhibitory effects as SNHG7-003 in AS. It was also discovered to be involved in the regulatory effects of the SNHG7-003/miR-1306-5p axis in VSMCs. On the whole, the findings of the present study indicate that SNHG7-003 may inhibit the proliferation, migration and invasion of VSMCs via the miR-1306-5p/SIRT7 signaling pathway. These findings may provide a novel basis for the development of treatment strategies for AS.

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Introduction

Atherosclerosis (AS) is a chronic cardiovascular disease characterized by high morbidity and mortality rates worldwide. AS can lead to the thickening and hardening of the walls of arteries, and to the formation of atherosclerotic plaques containing immune cells, mesenchymal cells and lipids. Once the unstable atherosclerotic plaques are ruptured, this may then contribute to thrombosis and may interrupt blood flow. At present, it has been found that the formation of atherosclerotic plaques involves multiple factors, including the presence of lipid metabolism disorders, vascular endothelial cell injury, vascular smooth muscle cell (VSMC) proliferation, platelet adhesion and others (1). VSMC proliferation has been revealed to play a pivotal role in the pathogenesis of AS, as VSMCs are closely associated with the key initiating factor, oxidized low-density lipoprotein (ox-LDL), which induces the phenotypic transformation of contractile VSMCs into synthetic VSMCs (2,3). Synthetic VSMCs proliferate, migrate and produce collagen more easily than contractile VSMCs (4,5), thus accelerating the progression of AS. Thus, it remains of utmost priority to identify the molecular mechanisms regulating the abnormal proliferation and migration of VSMCs.

Thousands of non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs), microRNAs (miRNAs/miRs) and small interfering RNAs (siRNAs), have been identified in the human genome (6). Each type of ncRNA has been identified to be involved in the progression of numerous types of disease, which provides a potential promising approach for the treatment of AS (7-10). lncRNAs are ncRNAs of >200 bp in length (11). A previous study revealed that lncRNAs played an important role in regulating the proliferation and migration of VSMCs (12-14). In particular, the lncRNA, small nucleolar RNA host gene 7-003 (SNHG7-003), has been observed to inhibit the

lipopolysaccharide-induced activation of the NF-κB signaling pathway and regulate the inflammatory response in human monocytes and macrophages, which were downregulated in the blood of patients with coronary artery disease with unstable plaques; it was therefore indicated to be a potential novel lncRNA biomarker for the diagnosis of unstable plaques (15).

Additional previous studies have indicated that miR-1306-5p may play a role in numerous types of cardiovascular disease. For example, miR-1306 expression levels were discovered to be upregulated in a miRNA expression profile of AS obliterans samples (16-18). Furthermore, a previous study by the authors confirmed that sirtuin 7 (SIRT7) modulated VSMC proliferation and migration via the Wnt/β-Catenin signaling pathway (19), and binding sites between miR-1306-5p and SIRT7 were predicted using the StarBase v2.0 database. However, to the best of our knowledge, the effects of SNHG7-003, miR-1306-5p and SIRT7 on VSMC have not yet been studied and remain unknown. Thus, the present study aimed to investigate the role of SNHG7-003 in VSMC proliferation and migration, as well as to clarify the potential underlying mechanisms.

Materials and methods

Cells, cell culture and treatment. VSMCs were obtained from the China Center for Type Culture Collection. VSMCs were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), and maintained in a humidified incubator with 95% air and 5% CO_2 at 37˚C. When cells grew to a certain density, cells in logarithmic phase were digested and passaged with 0.25% trypsin-EDTA (Gibco; Thermo Fisher Scientific, Inc.). Referring to a previous study (19), to establish the cell model of AS, VMSCs were incubated with 25, 50 and 100 μg/ml ox-LDL (Yiyuan Biotechnologies) for 24 h and the most suitable concentrations of ox-LDL were screened.

Bioinformatics prediction. The Starbase v2.0 website (http://starbase.sysu.edu.cn) was used to predict the potential miR-lncRNA binding partners and the downstream target genes of these miRs.

Cell transfection. The pcDNA-SNHG7-003 (SNHG7-003) and its blank control pcDNA-NC (vector) were constructed by Shanghai GenePharma Co. Ltd. The miR-1306-5p mimic and miR-NC were purchased from Guangzhou RiboBio Co.Ltd. The SIRT7 overexpression plasmid (SIRT7) and overexpression negative control (OE-NC) were obtained from BioVector. VSMCs in the logarithmic growth phase were seeded into 6-well plates and upon reaching 60-70% confluence, were transfected with the vectors or mimics and the respective NCs at a final concentration of 100 nM using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) for 8 h, according to the manufacturer's protocol. The transfection efficiency was determined using reverse transcription-quantitative PCR (RT-qPCR) or western blot analysis after 48 h. Cells were randomly assigned to the following groups: The control group, ox-LDL group, ox-LDL + vector group, ox-LDL + SNHG7-003 group, ox-LDL + SNHG7-003 + miR-NC group, ox-LDL + SNHG7-003 + miR-1306-5p group, ox-LDL + SNHG7-003 + miR-1306-5p + OE-NC group and ox-LDL + SNHG7-003 + miR-1306-5p + SIRT7 group.

RT-qPCR. Total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed into cDNA using the QuantiTect RT kit (Qiagen), according to the manufacturer's protocol. qPCR was subsequently performed using an Applied Biosystems 7500 Real-Time PCR system (Thermo Fisher Scientific, Inc.). The following primers were used for the qPCR: SNHG7-003 forward, 5'-CTTCGCCTGTGATGGACTTC-3' and reverse, 5'-CCTGCCCATCCCTTTATTCC-3'; and GAPDH forward, 5'-CTCACCGGATGCACCAATGTT-3' and reverse, CGC GTTGCTCACAATGTTCAT-3'. Total RNA was extracted and reverse transcribed into cDNA using the miRNAeasy Mini kit and miScript II RT kit (Qiagen GmbH), respectively. miR-1306-5p expression levels were detected using specific primers for miRNAs (Qiagen GmbH). The mRNA and miRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (20) and normalized to GAPDH or the small nuclear RNA U6, respectively as previously described.

Cell viability assay. A Cell Counting kit-8 (CCK-8; Beyotime Institute of Biotechnology, Inc.) assay was used to analyze the proliferation of the VSMCs, according to the manufacturer's protocol. Briefly, at 24 h post-transfection, the VSMCs were plated into a 96-well plate at a density of $2x10^3$ cells/well. Following 24 h of incubation, 10 μl CCK-8 solution were added to each well and incubated for further 4 h. A microplate reader (Bio-Rad, Laboratories, Inc.) was used to measure the absorbance at 450 nm.

Wound-healing assay. A wound-healing assay was used to analyze the migratory ability of the VSMCs. Briefly, $1x10^5$ transfected VSMCs were seeded into 12-well plates containing RPMI-1640 medium without FBS and were cultured to 80% confluence. Subsequently, the cell monolayer was scratched with a 200-μl plastic pipette tip. The cells were washed 3 times with PBS to remove the suspended cells prior to incubation in serum-free medium in a 12-well plate for 24 h in an incubator. The wound was photographed (magnification, x200) at 0 and 24 h using an inverted microscope (Olympus Corporation) and the extent of wound closure was semi-quantified using ImageJ software 2.0 (National Institutes of Health) at 0 and 24 h.

Transwell assay. The cell invasive ability was analyzed using a Transwell chamber (Corning, Inc.). Briefly, $1x10^6$ transfected cells suspended in free-serum medium were seeded into the upper chamber of the plate pre-coated with Matrigel (Corning, Inc.) for 30 min at 37˚C (2 mg/ml; 15 μl). RPMI-1640 medium supplemented with 10% FBS was added to the lower chambers. Following incubation for 24 h at 37˚C, the invasive cells were fixed with methanol and stained with 0.1% crystal violet (Sigma Aldrich; Merck KGaA) for 30 min at room temperature. Finally, the cells were visualized under a light microscope (magnification, x100; Olympus Corporation).

Western blot analysis. Total protein was extracted from the cells using RIPA lysis buffer (Beyotime Institute of Biotechnology), according to the manufacturer's protocol.

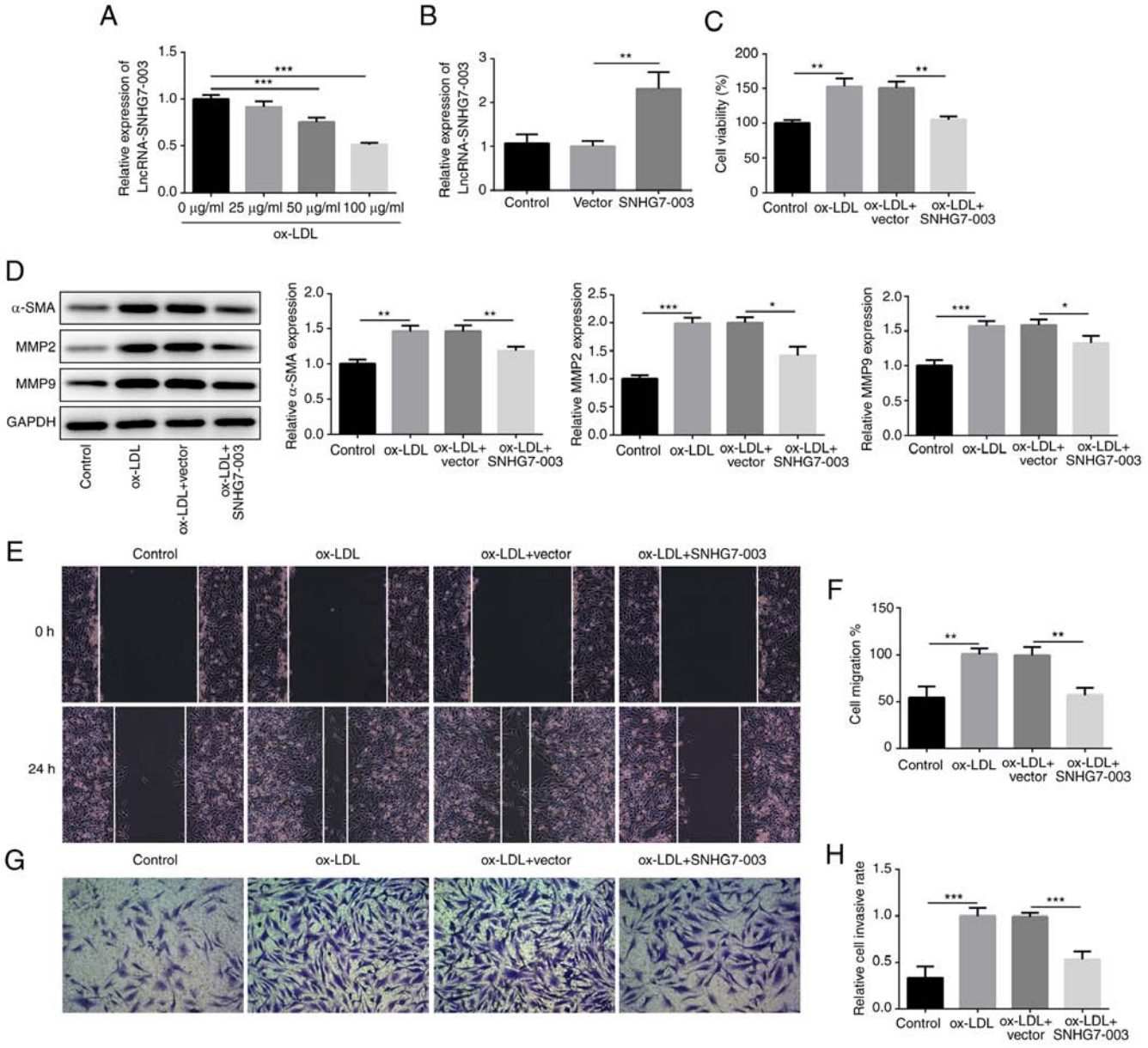

Figure 1. Overexpression of SNHG7-003 inhibits the proliferation, migration and invasion of VSMCs. (A) The expression level of SNHG7-003 in VSMCs stimulated with ox-LDL (0, 25, 50 and 100 μg/ml) was measured by RT-qPCR. (B) The transfection efficiency was evaluated by RT-qPCR. (C) CCK-8 assay was employed to assess cell viability. (D) The protein levels of α-SMA, MMP2 and MMP9 were measured by western blot analysis. (E and F) Wound-healing assay was conducted to assess the cell migration; magnification, x100. (G and H) Transwell assay was performed to estimate the capability of cell invasion; magnification, x100. *P<0.05, **P<0.01, ***P<0.001. SNHG7-003, small nucleolar RNA host gene 7-003; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein; MMP, matrix metalloproteinase; α-SMA, α smooth muscle actin.

Total protein was quantified using the BCA method and the protein samples were separated via 10% SDS-PAGE (Beyotime Institute of Biotechnology). A total of 30 μg separated protein/lane was subsequently transferred onto PVDF membranes (EMD Millipore) for different periods of time according to the different molecular weight of the proteins and then blocked with 5% skimmed milk overnight at 4˚C. The membranes were incubated at 4˚C overnight with the following primary antibodies: Anti-α-SMA (1:1,000; cat. no. 19245; Cell Signaling Technology, Inc.), anti-MMP-2 (1:1,000; cat. no. 40994; Cell Signaling Technology, Inc.), anti-MMP-9 (1:1,000; cat. no. 13667; Cell Signaling Technology, Inc.), anti-SIRT7 (1:1,000; cat. no. 5360; Cell Signaling Technology, Inc.) and anti-GAPDH (1:1,000; cat. no. 5174; Cell Signaling Technology, Inc.). Following incubation with the primary antibodies, the membranes were incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:5,000; cat. no. A0208; Beyotime Institute of Biotechnology) for 1 h at room temperature. Protein bands were visualized using an ECL detection kit (Bio-Rad Laboratories, Inc.) and the expression levels were analyzed using Image Lab software (Bio-Rad Laboratories, Inc.).

Dual luciferase reporter assay. SNHG7-003-wild-type (WT) and SNHG7-003-mutant (Mut), SIRT7-WT and SIRT7-Mut vectors were obtained from Promega Corporation. SNHG7-003-WT was constructed using the luciferase reporter vector containing SNHG7-003 sequences and the predicted

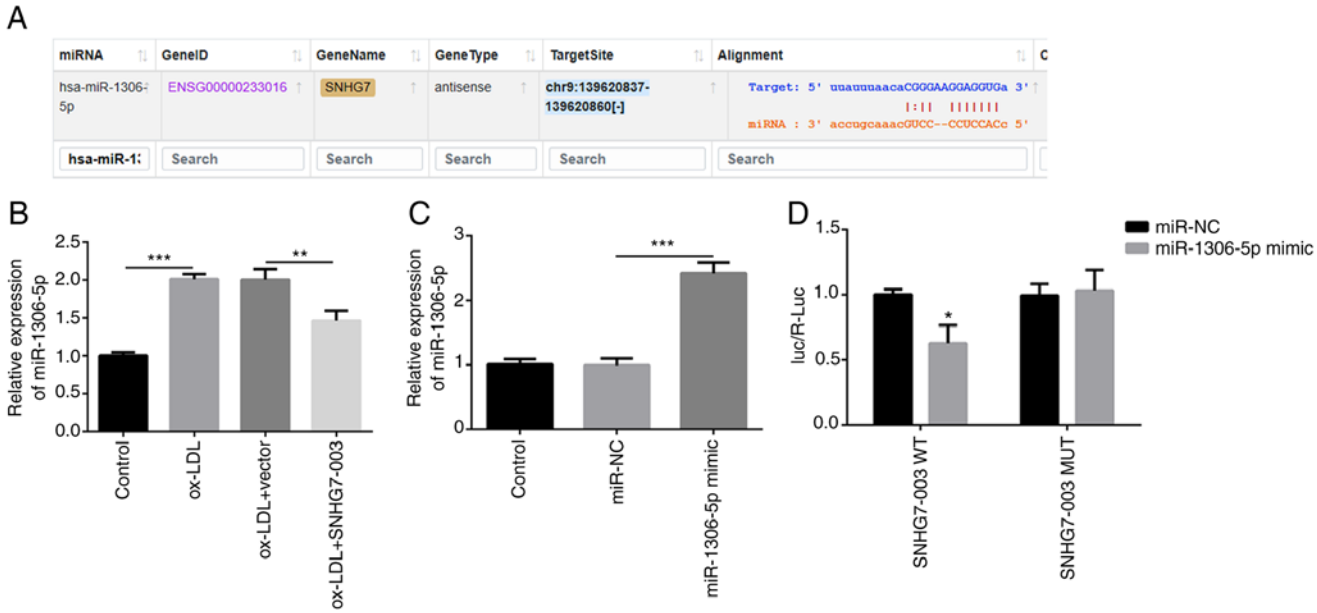

Figure 2. miR-1306-5p is the direct target of SNHG7-003. (A) Binding site between SNHG7-003 and miR-1306-5p was predicted by bioinformatics. (B) The expression of miR-1306-5p was examined in VSMCs transfected with pcDNA-vector or pcDNA-SNHG7-003 by RT-qPCR. (C) The expression of miR-1306-5p was examined in VSMCs transfected with miR-NC or miR-1306-5p mimic by RT-qPCR. (D) Relative luciferase activity was determined by luciferase reporter assay in VSMCs co-transfected with SNHG7-003 WT or SNHG7-003 Mut and miR-1306-5p or miR-NC. *P<0.05, **P<0.01, ***P<0.001. SNHG7-003, small nucleolar RNA host gene 7-003; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein.

miR-1306-5p binding site. SNHG7-003 with a Mut sequence was synthesized and subcloned into the vector, yielding SNHG7-003-Mut. The construction of the SIRT7-WT and SIRT7-Mut vectors was identical to the protocol described above. Subsequently, VSMCs were co-transfected with 50 ng SNHG7-003-WT/Mut (or SIRT7-WT/Mut) and 200 ng miR-1306-5p mimic or miR-NC using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the provided protocol. The relative luciferase activity was measured using a Dual Luciferase Reporter assay system (Promega Corporation) at 48 h following transfection. Firefly luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. Statistical analysis was performed using SPSS 22.0 software (IBM Corp.) and data are presented as the means ± SD of ≥3 experiments. A Student's t-test or one-way ANOVAs followed by a Tukey's test were used to determine statistical differences between groups. P<0.05 was considered to indicate a statistically significant difference.

Results

Overexpression of SNHG7-003 inhibits the proliferation, migration and invasion of VSMCs. A previous study reported that the expression levels of SNHG7-003 were downregulated in patients with coronary artery disease and unstable plaques (15). In the present study, to determine the role of SNHG7-003 in AS, VSMCs were stimulated with ox-LDL at various concentrations (0, 25, 50 and 100 μg/ml) for 24 h. The results revealed that the expression levels of SNHG7-003 were downregulated in a dose-dependent manner, with a significant difference being observed following treatment with the concentration of 50 or 100 μg/ml (Fig. 1A). The expression level of SNHG7-003 was the lowest in VSMCs exposed to ox-LDL at the higher concentration (100 μg/ml). Thus the concentration of 100 μg/ml ox-LDL was selected for use in the subsequent experiments. Subsequently, VSMCs were stimulated with 100 μg/ml ox-LDL for 24 h for subsequent experiments. The pcDNA-SNHG7-003 vector was constructed and the overexpression efficiency was verified by RT-qPCR; VSMCs transfected with pcDNA-SNHG7-003 exhibited upregulated expression levels of SNHG7-003 compared with the control vector (Fig. 1B). Subsequently, the roles of SNHG7-003 in VSMC proliferation and migration were investigated. The OE of SNHG7-003 reduced the increased proliferative ability of VSMCs induced by ox-LDL (Fig. 1C), and the observed downregulated expression levels of the VSMC-specific marker protein, α-SMA, following SNHG7-003 OE also validated this finding (Fig. 1D).

MMPs are known to decompose extracellular matrix proteins, consequently stimulating VSMC migration. As shown in Fig. 1D, the protein expression levels of MMP2 and MMP9 were upregulated by ox-LDL, whereas the OE of SNHG7-003 abrogated the effects induced by ox-LDL. The results of wound-healing and Transwell assays both demonstrated that ox-LDL stimulation promoted cell migration and invasion, respectively, whereas, these effects were impeded by the pcDNA-SNHG7-003 vector (Fig. 1E-H). These findings suggest that SNHG7-003 overexpression inhibits the proliferation, migration and invasion of VSMCs stimulated by ox-LDL.

miR-1306-5p is a direct target of SNHG7-003. First, the target genes of SNHG7-003 were predicted using the Starbase

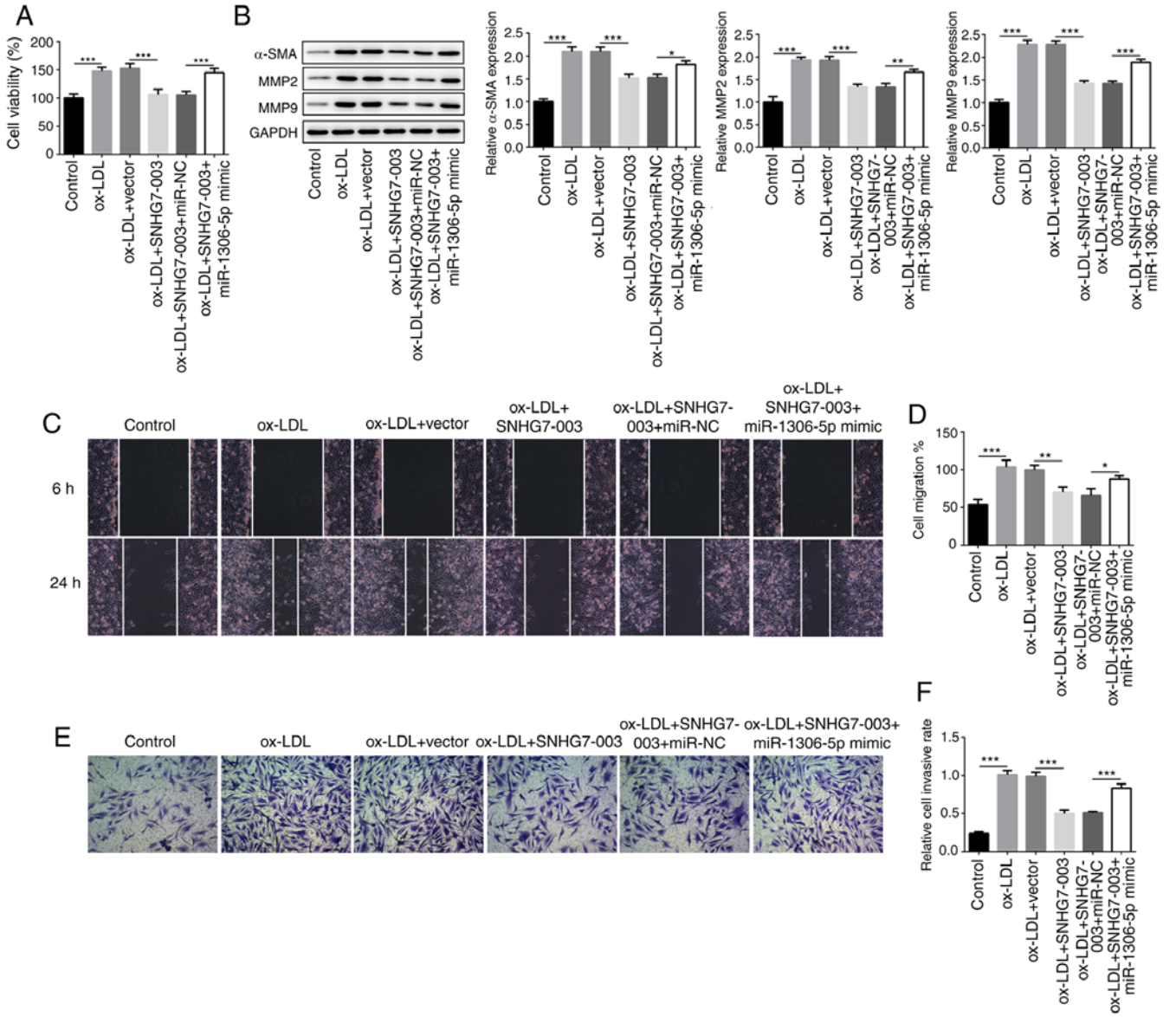

Figure 3. miR-1306-5p involves in the effects of SNHG7-003 on VSMCs. (A) The cell viability was measured by CCK-8 assay. (B) The protein levels of α-SMA, MMP2 and MMP9 were measured by western blot analysis. (C and D) Wound healing assay was conducted to assess the cell migration; magnification, x100. (E and F) Transwell assay was performed to estimate the capability of cell invasion; magnification, x100. *P<0.05, **P<0.01, ***P<0.001. SNHG7-003, small nucleolar RNA host gene 7-003; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein; MMP, matrix metalloproteinase; α-SMA, α smooth muscle actin.

v2.0 website (http://starbase.sysu.edu.cn). Through target gene screening and an extensive literature research (16-19), miR-1306-5p was considered to be the best candidate target gene of SNHG7-003 (Fig. 2A). Thus, the expression levels of miR-1306-5p in VSMCs were investigated in the present study. The results revealed that the miR-1306-5p expression levels were upregulated by ox-LDL. However, in the VSMCs transfected with pcDNA-SNHG7-003, the expression levels of miR-1306-5p were downregulated (Fig. 2B). Subsequently, miR-1306-5p mimic was transfected into the VSMCs to determine the expression levels of miR-1306-5p. As shown in Fig. 2C, miR-1306-5p mimic markedly upregulated the expression levels of miR-1306-5p. Subsequently, SNHG7-003-WT or SNHG7-003-Mut vectors were synthesized and co-transfected into the VSMCs with either the miR-1306-5p mimic or miR-NC. Co-transfection with the miR-1306-5p mimic markedly decreased the relative luciferase activity of the VSMCs transfected with SNHG7-003-WT, while it had no effect on the relative luciferase activity of the cells transfected with the SNHG7-003-Mut vector (Fig. 2D). Thus, these results suggest that SNHG7-003 targets and modulates the expression of miR-1306-5p.

miR-1306-5p is involved in the effects of SNHG7-003 on VSMCs. The present study subsequently aimed to determine the role of miR-1306-5p in the SNHG7-003-regulated proliferation and migration of VSMCs. While SNHG7-003 inhibited the proliferation of VSMCs, this effect was counteracted by transfection with miR-1306-5p mimic (Fig. 3A). Furthermore, the expression levels of α-SMA, MMP2 and MMP9 were also investigated in cells transfected with pcDNA-SNHG7-003 and/or miR-1306-5p mimic.

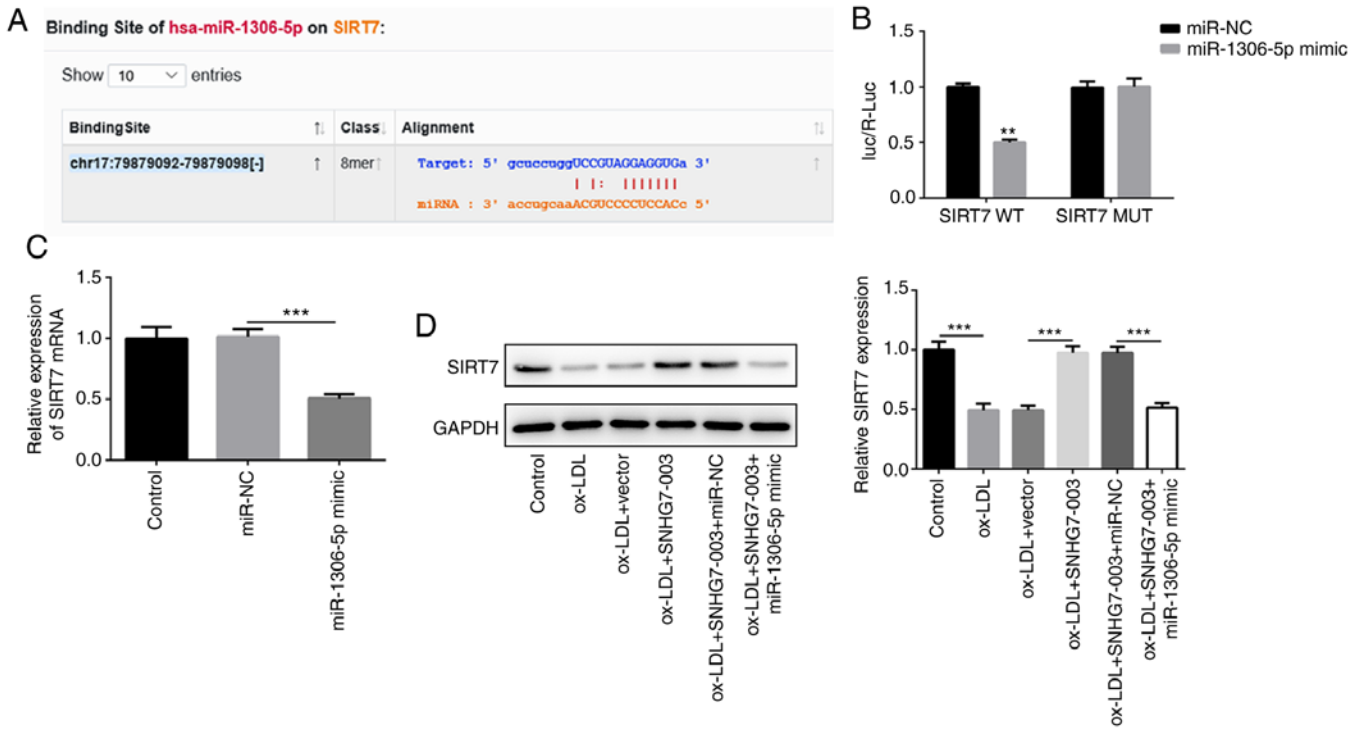

Figure 4. SIRT7 is the target gene of miR-1306-5p in VSMCs. (A) Complementary sequences between miR-1306-5p and SIRT7 were found using publicly available algorithms. The binding sites were predicted via Starbase v2.0. (B) Relative luciferase activity was determined by luciferase reporter assay in VSMCs co-transfected with SIRT7 WT or SIRT7 Mut and miR-1306-5p or miR-NC. (C) The mRNA expression of SIRT7 was examined in VSMCs transfected with miR-1306-5p or miR-NC by RT-qPCR. (D) The protein level of SIRT7 was estimated via western blot analysis. **P<0.01, ***P<0.001. SNHG7-003, small nucleolar RNA host gene 7-003; VSMCs, vascular smooth muscle cells; SIRT7, sirtuin 7; ox-LDL, oxidized low-density lipoprotein.

Transfection with miR-1306-5p mimic was found to reverse the downregulated expression levels of α-SMA, MMP2 and MMP9 mediated by pcDNA-SNHG7-003 (Fig. 3B). Similar trends were observed following the analysis of VSMC migration and invasion (Fig. 3C-F). Taken together, these data suggest that miR-1306-5p inhibits the effects of SNHG7-003 on the proliferation, migration and invasion of VSMCs.

SIRT7 is the target gene of miR-1306-5p in VSMCs. To further elucidate the underlying mechanisms through which the SNHG7-003/miR-1306-5p axis modulates VSMC proliferation and migration, the StarBase v2.0 website was used to search for miR-1306-5p target genes and the binding sites of miR-1306-5p and SIRT7 were predicted. Following a comprehensive comparison, SIRT7 was considered to be the best candidate target gene of miR-1306-5p (Fig. 4A). SIRT7-WT or SIRT7-Mut were then constructed and co-transfected with miR-1306-5p mimic or miR-NC into VSMCs. The results of the dual luciferase reporter assay validated that miR-1306-5p could directly bind to SIRT7 (Fig. 4B). Consequently, the OE of miR-1306-5p down-regulated the mRNA expression levels of SIRT7 (Fig. 4C). Notably, the downregulated expression levels of SIRT7 in ox-LDL-stimulated VSMCs were reversed following transfection with pcDNA-SNHG7-003, while transfection with miR-1306-5p mimic weakened the effects of SNHG7-003 on SIRT7 (Fig. 4D). Overall, these results suggested that SIRT7 may be a target gene of miR-1306-5p and may be regulated by SNHG7-003.

SNHG7-003/miR-1306-5p/SIRT7 axis is involved in VSMC proliferation, migration and invasion. To further elucidate whether the underlying mechanisms of VSMC proliferation and migration are associated with SIRT7, OE-SIRT7 or OE-NC vectors were constructed and transduced into VSMCs successfully, which was demonstrated by the upregulated expression levels of SIRT7 at the mRNA and protein level (Fig. 5A and B). However, following transfection with the OE-SIRT7 vector, the proliferative ability of the cells was markedly inhibited, preventing the synergism of SNHG7-003 and miR-1306-5p on VSMCs simulated by ox-LDL (Fig. 5C). The expression levels of α-SMA, MMP2 and MMP9 were also analyzed and the results revealed that OE-SIRT7 attenuated the effects of the SNHG7-003/miR-1306-5p axis on VSMC proliferation and migration (Fig. 5D). A similar trend was observed upon analyzing the VSMC migratory and invasive abilities (Fig. 5E-H). In summary, these data indicate that the SNHG7-003/miR-1306-5p/SIRT7 axis may be involved in the proliferation, migration and invasion of VSMCs.

Discussion

AS is a chronic degenerative disease commonly accompanying numerous types of cardiovascular disease, with its pathogenesis being closely associated to the aberrant proliferation and migration of VSMCs (21). Accumulating evidence has reported that ncRNAs served a significant role in AS progression, which suggested their potential to be developed into therapeutic agents and targets for AS (22,23). For example, the lncRNA LEF1-AS1 has been discovered to

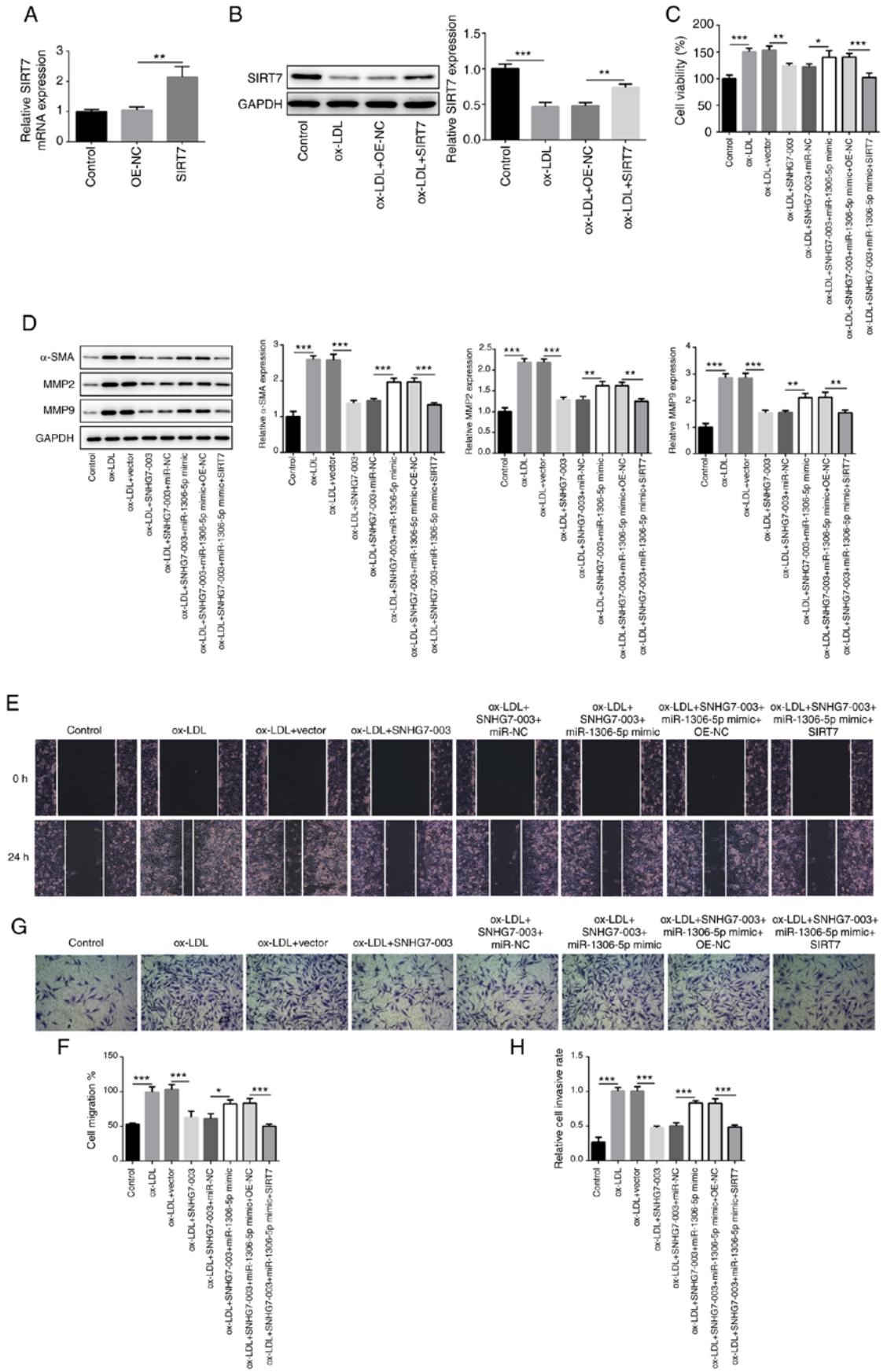

Figure 5. SNHG7-003/miR-1306-5p/SIRT7 axis plays a role in VSMCs proliferation, migration and invasion. (A) The mRNA expression of SIRT7 was examined in VSMCs transfected with OE- SIRT7 or OE-NC by RT-qPCR. (B) The protein level of SIRT7 in VSMCs transfected with OE- SIRT7 was estimated by western blot analysis. (C) Cell viability was measured by CCK-8. (D) The protein levels of α-SMA, MMP2 and MMP9 were measured by western blot analysis. (E and F) Wound-healing assay was conducted to assess the cell migration; magnification, x100. (G and H) Transwell assay was performed to estimate the capability of cell invasion; magnification, x100. *P<0.05, **P<0.01, ***P<0.001. SNHG7-003, small nucleolar RNA host gene 7-003; VSMCs, vascular smooth muscle cells; SIRT7, sirtuin 7; ox-LDL, oxidized low-density lipoprotein; MMP, matrix metalloproteinase; α-SMA, α smooth muscle actin.

regulate the migration and proliferation of VSMCs through the miR-544a/PTEN axis (24). Another study revealed that the lncRNA ENST00000602558.1 mediated ABCG1 expression levels and the cholesterol efflux to HDL from VSMCs via binding to p65 (25). In addition, the lncRNA SNHG16 has been found to promote the proliferation and inflammatory response of macrophages through miR-17-5p/NF-κB in patients with AS (26). These reports highlighted the significant role that ncRNAs play in AS. However, at present, the role of lncRNAs in AS pathogenesis is still poorly studied, and further research is required to fully understand the function of lncRNAs in AS.

SNHG is a class of lncRNA that plays an important role in biological regulation of malignant tumors. The abnormal expression of SNHG has been detected in several types of tumors gastric cancer and breast cancer and is closely associated with the occurrence and development of tumors (27,28). The most widely studied of these is SNHG7. SNHG7 is located on chromosome 9 and the average length of these transcripts was 2,176 bp, which can guide the post-translational modification of small nucleolar RNA (snoRNA), while snoRNA can directly participate in the progression of cancer (29). A number of studies have demonstrated that SNHG7 promotes the proliferation, migration and invasion of multiple tumor cells, including malignant pleural mesothelioma, chromophobe renal cell carcinoma and lung cancer (30-32). However, to the best of our knowledge, there are no studies available to date on the role of SNHG7 in AS. Notably, a previous study used RNA-Seq to screen out differentially expressed lncRNAs between a stable plaque group and the unstable plaque, and the expression levels in the blood samples from each group were determined; it was identified that SNHG7-003 expression in AS plaques was decreased significantly, and SNHG7-003 suppressed the NF-κB signaling pathway and reduced the secretion of inflammatory factors, suggesting that blood SNHG7-003 may be a potential biomarker for patients with coronary artery diseases (15). In view of the above-mentioned studies, it was hypothesized that SNHG7-003 may act as a tumor suppressor gene in the development of AS. In the present study, ox-LDL was used to induce VSMC proliferation and it was discovered that the expression levels of SNHG7-003 were downregulated following ox-LDL treatment in a dose- and time-dependent manner, while the overexpression of SNHG7-003 counteracted the promoting effects of ox-LDL on cell proliferation and migration.

lncRNAs can exert their biological functions by competitive binding with miRNA to inhibit the negative regulation of miRNA on target genes (33,34). Therefore, the authors aimed to further study the regulatory mechanisms of SNHG7-003 in AS. The Starbase v2.0 website predicted several miRNAs that may target SNHG7-003 and miR-1306-5p was determined as a target following screening. By searching the literature, it was found that miR-1306-5p was involved in numerous types of cardiovascular diseases and that miR-1306 was specifically upregulated in AS obliterans samples (16-18). These results suggested that miR-1306-5p may play an important role in the pathogenesis of AS; however, the role of miR-1306-5p in AS has not been studied to date, at least to the best of our knowledge. Thus, the present study investigated the association between miR-1306-5p and SNHG7-003. The results of dual-luciferase reporter assay revealed that SNHG7-003 acted as a sponge towards miR-1306-5p in VSMCs. miR-1306-5p overexpression reversed the inhibitory effects of SNHG7-003 overexpression on the proliferation, migration and invasion of VSMCs induced by ox-LDL. These results revealed that SNHG7-003 inhibited the proliferation and metastasis of VSMCs by downregulating miR-1306-5p.

In order to construct a complete regulatory axis, we once again predicted the target gene SIRT7 for miR-1306-5p by Starbase v2.0 website. SIRT7 is the full-length 1.7-kb mRNA located on chromosome 17 and belongs to classIII of histone deacetylase, which is widely involved in a variety of cell progression, including cell cycle, inflammation and apoptosis (35). Previous study showed that SIRT7 plays a role served as a tumor suppressor or oncogene in different tumors or diseases (36). For example, SIRT7 is highly expressed in gastric cancer, and the expression level of SIRT7 is correlated positively with the severity of the disease (37). In papillary thyroid carcinoma, SIRT7 promotes tumorigenesis via activating the Akt pathway by targeting DBC1 (38). In breast cancer, SIRT7 inhibits the lung metastasis of breast cancer by suppressing epithelial-mesenchymal transition (39). However, to date, there are limited studies available on SIRT7 in non-neoplastic diseases, at least to the best of our knowledge. A previous study demonstrated that the SIRT7 expression levels were downregulated in VSMCs stimulated with ox-LDL and that SIRT7 regulated VSMC proliferation and migration via the Wnt/β-Catenin signaling pathway (19). However, the study of SIRT7 in AS warrants further in-depth investigations. Thus, in the present study, SIRT7 overexpression plasmids were synthesized, and functional experiments were performed. The expression of SIRT7 was downregulated in VMSCs cells stimulated with ox-LDL. SIRT7 overexpression abrogated the effects of the SNHG7-003/miR-1306-5p axis on VSMC proliferation, migration and invasion. However, there were several limitations to the present study. Firstly, the specific mechanisms of SNHG7-003 regulating the miR-1306/SIRT7 pathway require further investigations. Secondly, the present study only performed *in vitro* cellular experiments, and animal experiments are required to verify the results of the current study. In the future, the authors aim to conduct an in-depth study on the *in vivo* functions and clinical relevance of SNHG-003.

In conclusion, the findings of the present study indicated that the expression levels of SNHG7-003 were downregulated in ox-LDL-stimulated VSMCs. SNHG7-003 was identified to be able to directly target miR-1306-5p, which proceeded to affect the expression levels of the downstream target gene, SIRT7, exerting an inhibitory effect on cell proliferation, migration and invasion. Thus, it was suggested that SNHG7-003 may inhibit the proliferation and migration of VSMCs through the miR-1306-5p/SIRT7 pathway, and plays a protective role in the progression of AS. The results enrich the biological function of SNHG7-003 in the progression of AS, provide a new molecular mechanism through which SNHG7-003 regulated AS, and promote

the development of lncRNA-directed diagnostics and therapeutics against AS.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Authors' contributions

JZ, QT and HC contributed to the acquisition of data. KC, HW and ZC contributed to the analysis and interpretation of data, YX and HY contributed to the conception and design of the study. KL and YL performed the research. All authors read and approved the final version of the manuscript to be published.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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