

Original Article

lncRNA H19 facilitates vascular neointima formation by targeting miR-125a-3p/FLT1 axis

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Abstract

The aberrant proliferation and migration of vascular smooth muscle cells (VSMCs) contribute to the development of neointima formation in vascular restenosis. This study aims to explore the function of the long noncoding RNA H19 in neointima formation. A mouse carotid ligation model was established, and human vascular smooth muscle cells (VSMCs) were used as a cell model. lncRNA H19 overexpression promoted VSMC proliferation and migration. Moreover, miR-125a-3p potentially bound to lncRNA H19, and Fms-like tyrosine kinase-1 (FLT1) might be a direct target of miR-125a-3p in VSMCs. Upregulation of miR-125a-3p alleviated lncRNA H19-enhanced VSMC proliferation and migration. Furthermore, rescue experiments showed that enhanced expression of miR-125a-3p attenuated lncRNA H19-induced FLT1 expression in VSMCs. In addition, the overexpression of lncRNA H19 significantly exacerbated neointima formation in a mouse carotid ligation model. In summary, lncRNA H19 stimulates VSMC proliferation and migration by acting as a competing endogenous RNA (ceRNA) of miR-125a-3p. lncRNA H19 may be a therapeutic target for restenosis.

Key words vascular smooth muscle cell, neointima formation, lncRNA H19, miR-125a-3p, FLT1

Introduction

Cardiovascular disease is the main cause of mortality globally. With the development of endovascular surgery, the prognosis of patients with vascular disease has improved. However, restenosis after angioplasty or stenting remains a major clinical problem [1,2]. The proliferation and migration of vascular smooth muscle cells (VSMCs) contribute to neointima formation, which leads to vascular restenosis [3–5]. Therefore, it is urgent to elucidate the molecular mechanism underlying neointima formation.

Recent studies have reported that long noncoding RNAs (lncRNAs) have various biological functions, such as transcrip-

tional regulation, protein degradation and chromatin stabilization [6,7]. Moreover, lncRNAs are involved in various diseases, such as cancer, neurological disease and heart disease [8–10]. In particular, the lncRNA H19 is located on chromosome 11 and promotes several types of cancer [11,12]. Sun *et al.* [13] reported that H19 knockdown may prevent atherosclerosis deterioration through increased p53-mediated VSMC apoptosis. In addition, lncRNA H19 promoted the proliferation of VSMCs in a miR-675-dependent manner [14]. Nevertheless, the role of lncRNA H19 in vascular neointima formation has not been well elucidated.

In this study, we found that lncRNA H19 overexpression

significantly promoted neointima formation *in vivo* and VSMC proliferation and migration *in vitro*. Mechanistically, we found that lncRNA H19 acted as a ceRNA of miR-125a-3p and facilitated the expression of the miR-125a-3p target FLT1 in VSMCs. We further demonstrated that lncRNA H19 knockdown significantly reduced the expression of FLT1. Moreover, overexpression of miR-125a-3p inhibited the proliferation and migration of VSMCs by targeting FLT1.

Materials and Methods

Animals

Animal experiments were performed at the Experimental Animal Center of Gannan Medical University following the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Ethics Committee of Animal Experiments of Gannan Medical University (Ethics No. 2022015). Male C57BL/6 mice (8–10 weeks old) were obtained from the Experimental Animal Center of Gannan Medical University. For the carotid ligation model, male C57BL/6 mice were anesthetized with an intraperitoneal injection of 2% pentobarbital sodium (0.3 mL per 100 g), and the right carotid artery was ligated with 8-0 thread. A dissecting microscope was used to monitor the procedure. Postsurgery, the mice were injected subcutaneously with buprenorphine (0.05 mg/kg) to minimize pain and euthanized by cervical dislocation. The carotid arteries were harvested at 0 and 14 days after ligation, fixed in 4% formalin overnight and then stained with hematoxylin and eosin (H&E). Images were analyzed under a microscope (Leica, Wetzlar, Germany).

Sample acquisition

All human arteries were obtained from patients with lower extremity arteriosclerosis occlusion, and normal arteries were obtained from donors without arteriosclerosis occlusion at the First Affiliated Hospital of Sun Yat-sen University. These tissues were approved by donors or by their families. After dissection, the samples were immediately snap frozen in liquid nitrogen and stored at -80°C for qPCR or western blot analysis. Some samples were fixed in 10% formalin for embedding, sectioning and staining. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards (Ethics No. 2020518). Informed consent was obtained from all individual participants included in the study.

H&E staining

Arteries collected from mice were fixed in methacarn fixative solution and then embedded in paraffin as described previously [15]. Next, the paraffin was cut into 5 μm sections. After deparaffinization and rehydration, all sections were stained with H&E (Servicebio, Wuhan, China) following standard procedures. Finally, the sections were photographed and examined. The neointimal areas of the sections were analyzed by an independent investigator in a blinded manner using ImageJ software. The neointimal area was calculated by subtracting the luminal area from the area enclosed by the internal elastic lamina (IEL).

Cell culture

The human aortic vascular smooth muscle cell line T/G HA-VSMCs

(ATCC CRL-1999) and human embryonic kidney 293T cells (ATCC CRL-3216) were cultured in Dulbecco's modified Eagle's medium (DMEM)/Ham's F12 medium (Gibco, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS; PAN Biotech, Aidenbach, Germany) and 1% penicillin/streptomycin sulfate in a 5% CO_2 incubator at 37°C .

Fluorescence *in situ* hybridization (FISH)

Subcellular localization of lncRNA H19 was detected by a FISH kit (BersinBio, Guangzhou, China) according to the manufacturer's instructions. Paraffin sections of arteries and VSMCs were used. Paraffin sections were prepared as described above. The tissue sections were consistent with the vascular tissue used for H&E staining and were collected from sham and carotid artery balloon injury model rats. VSMCs were seeded on cell slides and fixed in 4% paraformaldehyde for 30 min at room temperature. After permeabilization, the sections and cells were prehybridized with hybridization solution and then incubated with the TAMRA-labeled lncRNA H19 oligonucleotide probe. After they were washed with saline sodium citrate (SSC) solution, the cell nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) for 5 min at room temperature. Fluorescence images were obtained by laser scanning confocal microscopy (LSM700; Carl Zeiss, Oberkochen, Germany).

Quantitative real-time polymerase chain reaction (qPCR)

Total RNA from arteries or VSMCs was isolated using TRIzol reagent (Aidlab Biotechnologies, Beijing, China) according to the manufacturer's protocol. Total RNA was then reverse transcribed using a miScript II RT Kit (Qiagen, New York, USA) for mRNA or a qScriptTM microRNA cDNA Synthesis Kit (Quanta BioSciences, Beverly, USA) for miRNA. PCR was performed using the miScript SYBR Green PCR Kit (Qiagen) with GAPDH or U6 as internal controls in the StepOnePlus system (Applied Biosystems, Waltham, USA). The fold change in mRNA or miRNA expression was calculated using the $2^{-\Delta\text{CT}}$ method. The sequences of primers used were as follows: lncRNA H19, forward 5'-GATGACAGGTGTGGTCAACG-3' and reverse 5'-CAGACATGAGCTGGGTAGCA-3'; miR-125a-3p, forward 5'-ACAGGTGAGGTTCTTGGGAGCC-3' and reverse 5'-AACGCTTCACGAATTTGCGT-3'; FLT1, forward 5'-ACAA CAGGACCATGCACCAT-3' and reverse 5'-GCAGTGCTCACCTCTAACGA-3'; GAPDH, forward 5'-TGCCACTCAGAAGACTGTG G-3' and reverse 5'-TTCAGCTCTGGGATGACCTT-3'; and U6, forward 5'-CTCGCTTCGGCAGCACA-3' and reverse 5'-AACGCTT CACGAATTTGCGT-3'.

Scratch wound healing assay

Cell migration was tested by the scratch wound healing assay. VSMCs (5×10^5 cells) were grown on 35 mm plates. A pipette tip was used to scrape the cell monolayer, and then a scratch wound was created in 2 perpendicular directions in the confluent cell layer. The medium was replenished immediately, and phase-contrast images of the cells were captured 48 h after the scratch.

Cell counting kit-8 (CCK-8) assay

A CCK-8 kit (Dojindo, Kumamoto, Japan) was used to determine the proliferation of VSMCs. Cells were seeded in 96-well culture plates (5×10^3 cells/well) and cultured in DMEM supplemented with 10% FBS for 24 h. According to the manufacturer's protocol, 10 μL of

CCK-8 reagent was added to each well, and the cells were incubated at 37°C for 2 h. The absorbance (optical density, OD) at 450 nm was measured on an enzyme immunoassay analyzer (Bio-Rad, Hercules, USA).

RNA antisense purification (RAP)

The association between lncRNA H19 and miR-125a-3p was identified by a RAP kit (BersinBio, Guangzhou, China) according to the manufacturer's instructions. Briefly, lncRNA H19 was pulled down from VSMC lysates by an antisense probe, and miR-125a-3p in the precipitates was detected by qPCR.

Subcellular localization of lncRNA H19

The subcellular localization of lncRNA H19 was validated by using a nuclear-cytoplasmic RNA separation kit (PARIS Kit; Invitrogen, Waltham, USA) according to the manufacturer's protocols.

Western blot analysis

Western blot analysis was performed as described previously [16]. Briefly, protein extracted from VSMCs was resolved by 8%–12% SDS-PAGE and transferred onto Immobilon-P membranes (Millipore, Shanghai, China). The membranes were incubated in 5% nonfat milk for 1 h and then incubated at 4°C with primary antibodies against FLT1 (diluted at 1:500; Proteintech, Wuhan, China) and GAPDH (diluted at 1:2500; Cell Signaling Technology) overnight. The membranes were then incubated with secondary antibodies (Proteintech) and detected with a SuperSignal West Femto Maximum Sensitivity Substrate kit (Thermo Fisher Scientific, Shanghai, China) according to the manufacturer's instructions.

Luciferase reporter assay

Bibiserv2 (<https://bibiserv.cebitec.uni-bielefeld.de/>) was used to predict the biological target genes of lncRNA H19 and miR-125a-3p. The cDNA sequence of lncRNA H19 and the 3'UTR sequence of FLT1 containing the binding sites for miR-125a-3p were cloned and inserted into the psiCHECKTM-2 luciferase reporter vector (GeneChem, Shanghai, China) to construct the lncRNA H19-wild type (WT) and FLT1 WT vectors. The corresponding lncRNA H19-mutant (Mut) and FLT1 (Mut) vectors were constructed based on the mutated binding sequences (GeneChem). Transfection was performed using Lipofectamine 2000 (Thermo Fisher Scientific) and Opti-MEM® I reduced serum medium (Gibco, Shanghai, China). After transfection for 48 h, the luciferase activities were measured by using a dual luciferase reporter assay system (Promega, Madison, USA) according to the manufacturer's protocol. Renilla luciferase activities were normalized to firefly luciferase activities, and the data are expressed as the fold change relative to the corresponding control group.

Complete ligation of the common carotid artery and lentiviral infection

Mice were anesthetized, and the right common carotid artery was ligated with an 8-0 propylene string through a midline neck incision. The ligated artery was washed with saline and then incubated with 100 µL of Pluronic F127 gel (25% w/v dissolved 0.9% saline) containing vector lentivirus or lncRNA H19 overexpression lentivirus (1×10^9 TU) for 20 min. Two weeks after ligation, the carotid arteries were excised and analyzed.

Lentivirus transfection of VSMCs

Lentiviruses carrying lncRNA H19, FLT1 or the control vector were used to infect VSMCs *in vitro*. The lentivirus was obtained from GeneChem (Shanghai, China). The vector lentivirus used is shown in [Supplementary Figure S1](#). The procedure for lentivirus packaging and infection was described previously [17,18]. Briefly, low-passage (P3–5) VSMCs at approximately 80% confluence were infected with lentiviral particles for 24–48 h. After infection, puromycin (2 µg/mL) was used to screen for and establish stable cell lines.

miRNA transfection

miRNA transfection was performed using Lipofectamine RNAiMAX (Thermo Fisher Scientific, Shanghai, China) and Opti-MEM® I reduced serum medium (Gibco, Shanghai, China). Briefly, low-passage (P3–5) VSMCs or HEK293 at approximately 80% confluence were transfected with NC or miR-125a-3p mimic for 24–48 h, and then attributed to different cell function assay. NC and miR-125a-3p mimic were obtained from GeneChem (Shanghai, China). The sequence of miR-125a-3p is 5'-UGCCAGUCUCUAGGUCCU GAGACCUUUAACCUGUGAGGACAUCCAGGGUCACAGGUGAGG UUCUUGGGAGCCUGGCGUCUGGCC-3'. The sequence of NC is 5'-U AGCCUGUACAAUGCUGCUGUU-3'.

Statistical analysis

All the data are reported as the mean ± standard deviation (SD). Statistical differences were analyzed by unpaired Student's *t* test for two groups and one-way ANOVA followed by Student-Newman-Keuls post hoc test for multiple groups. All the statistical analyses were conducted using SPSS 20.0 (SPSS Inc., Chicago, USA). *P* < 0.05 indicated statistical significance.

Results

lncRNA H19 was upregulated in vascular neointima formation

Based on our previous microarray data (GSE121839), lncRNA H19 was upregulated in rat balloon-injured arteries compared with that in sham control arteries [19]. To validate the upregulation of lncRNA H19 during vascular neointima formation, we established a mouse carotid ligation model ([Figure 1A](#)). Based on this model, we found that lncRNA H19 was dramatically elevated in injured carotid arteries and was mainly located in the cytoplasm of cells in the neointima ([Figure 1B](#)). qPCR analysis confirmed that lncRNA H19 expression was increased in ligation-injured arteries compared with that in sham arteries ([Figure 1C](#)). In addition, lncRNA H19 was significantly upregulated in arteriosclerosis obliterans (ASO) arteries compared with normal arteries of patients ([Figure 1D](#)).

lncRNA H19 promoted the proliferation and migration of VSMCs

To determine the role of lncRNA H19 in the regulation of VSMC proliferation and migration, we determined the effect of lncRNA H19 on the proliferation and migration of human VSMCs. After the overexpression of lncRNA H19 ([Figure 2A](#)), the proliferation of VSMCs increased compared with that of cells infected with the empty vector (Vector) ([Figure 2B](#)). Moreover, the overexpression of lncRNA H19 promoted VSMC migration ([Figure 2C,D](#)). The contractile proteins SM22 α and α -SMA inhibited the conversion of VSMCs from the contractile phenotype to the synthetic phenotype.

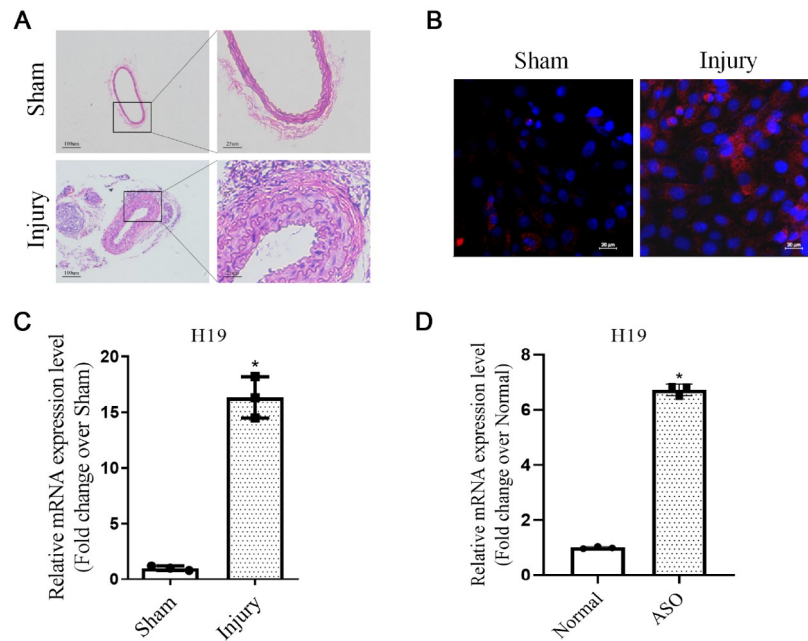


Figure 1. lncRNA H19 was upregulated in injured arteries (A) The establishment of the mouse carotid ligation model detected by H&E staining. Sham-operated mice and ligated mice. Scale bars: 100 μ m and 25 μ m. (B) FISH of lncRNA H19 (red) in paraffin-embedded sham carotid arteries and injured carotid arteries in mice. The nuclei were stained with DAPI (blue). Scale bar: 50 μ m. (C) Relative expression of lncRNA H19 in mouse carotid arteries detected by qPCR ($n=5$ independent experiments). (D) Relative expression of lncRNA H19 in human normal arteries and ASO arteries ($n=5$ independent experiments). All the data are presented as the mean $\pm$ SD. * $P<0.05$ versus the sham group or normal group (unpaired two-tailed Student's t test).

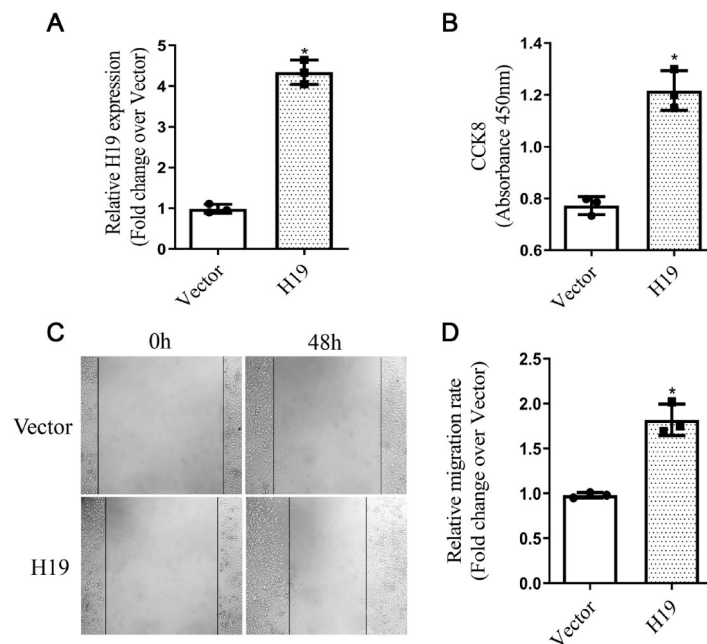


Figure 2. lncRNA H19 promoted VSMC proliferation and migration (A) Relative expression of lncRNA H19 in human VSMCs infected with lncRNA H19 lentivirus or vector lentivirus. * $P<0.05$ versus the vector group ($n=5$ independent experiments). (B) The proliferation of VSMCs infected with lncRNA H19 lentivirus or vector lentivirus was measured by a CCK-8 assay. * $P<0.05$ versus the vector group ($n=5$ independent experiments). (C) The migration of human VSMCs was evaluated by a wound healing assay. Magnification: 100 $\times$. (D) Quantitative analysis of the migration distance. * $P<0.05$ versus the vector group ($n=3$ independent experiments). All the data are presented as the mean $\pm$ SD (unpaired, two-tailed Student's t test).

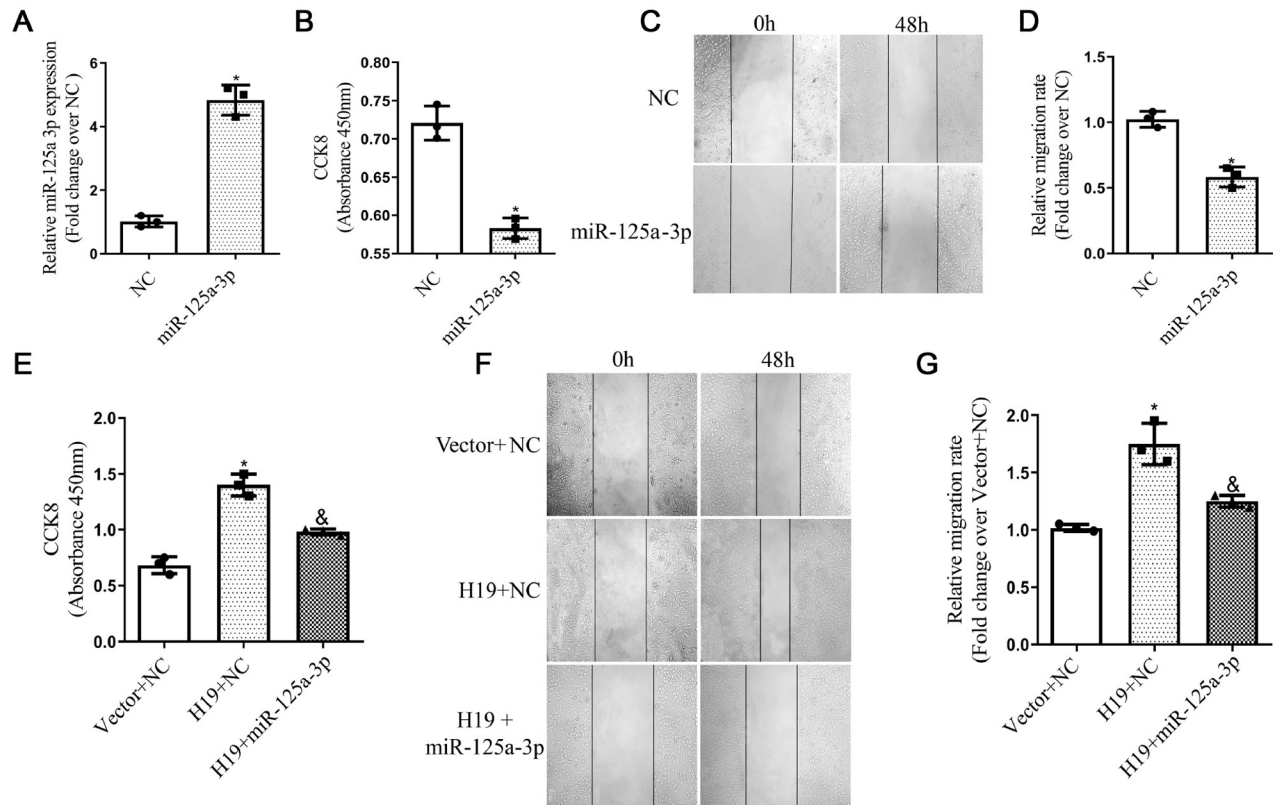


Figure 4. miR-125a-3p inhibited VSMC proliferation and migration (A) miR-125a-3p in human VSMCs treated with NC or miR-125a-3p mimics. * $P < 0.05$ versus the NC group ($n = 5$). (B) The proliferation of human VSMCs treated with NC or miR-125a-3p mimics was measured by a CCK8 assay. * $P < 0.05$ versus the NC group. (C) The migration of human VSMCs treated with NC or miR-125a-3p mimics. Magnification: $100\times$. (D) Quantitative analysis of the migration distance. * $P < 0.05$ versus the NC group ($n = 3$). (E) The proliferation of human VSMCs treated with vector+NC, H19+NC or H19+miR-125a-3p was measured by a CCK-8 assay. * $P < 0.05$ versus the Vector+NC group; & $P < 0.05$ versus the H19+NC group ($n = 5$). (F) The migration of human VSMCs treated with vector+NC, H19+NC or H19+miR-125a-3p. Magnification: $100\times$. (G) Quantitative analysis of the migration distance. * $P < 0.05$ versus the vector+NC group; & $P < 0.05$ versus the H19+NC group ($n = 3$).

promoted VSMC proliferation and migration (Figure 5H–L). Collectively, these data suggest that lncRNA H19 promotes VSMC proliferation and migration by inducing FLT1 expression by suppressing the function of miR-125a-3p.

lncRNA H19 promoted neointima formation after vascular injury *in vivo*

To confirm the role of lncRNA H19 in restenosis *in vivo*, we upregulated lncRNA H19 in the carotid artery by local incubation with lentivirus harboring lncRNA H19. The results showed that the overexpression of lncRNA H19 markedly inhibited miR-125a-3p and promoted FLT1 expression. Furthermore, lentiviral infection of injured carotid vascular walls with lncRNA H19 significantly increased neointima formation (Figure 6A–D).

Discussion

It is well known that restenosis after angioplasty or stenting is due to neointima formation, which is mainly caused by the aberrant proliferation and migration of VSMCs. To date, restenosis is still a troublesome clinical problem. Although drug-eluting stents (DESs) and drug-coated balloons (DCBs) significantly reduce the incidence of restenosis by releasing cytotoxic drugs, they inhibit re-endothelialization and cause late thrombosis reactions. Thus, it is urgent to explore effective targets to accurately suppress the proliferation and

migration of VSMCs with minimal side effects.

lncRNAs have been shown to be involved in both physiological and pathological processes and have become promising targets for treating different diseases [21]. lncRNA H19 is implicated in promoting tumor progression and aneurysm formation [22–26]. However, the role of lncRNA H19 in restenosis remains largely unknown. Our previous study showed that the lncRNA H19 was significantly upregulated in injured carotid arteries [19], which is consistent with the findings of another study [14]. This study confirmed that lncRNA H19 was increased in injured arteries and ASO arteries *in vivo* and in PDGF-BB-treated VSMCs *in vitro*. Moreover, we found that lncRNA H19 significantly promoted the proliferation and migration of VSMCs. These results indicate the pivotal role of lncRNA H19 in regulating VSMC function.

lncRNAs in the nucleus function by modulating transcription, while lncRNAs in the cytoplasm play a role in posttranscriptional control by serving as ceRNAs of microRNAs [27–29]. In this study, we found that lncRNA H19 was located mainly in the cytoplasm. We used bioinformatics analysis and found that miR-125a-3p may bind to lncRNA H19. Our previous study reported that the overexpression of miR-125a-3p significantly inhibited VSMC proliferation and migration [30]. Thus, we speculated that lncRNA H19 may act as a ceRNA of miR-125a-3p to regulate target gene expression and promote the proliferation and migration of VSMCs. Next, we

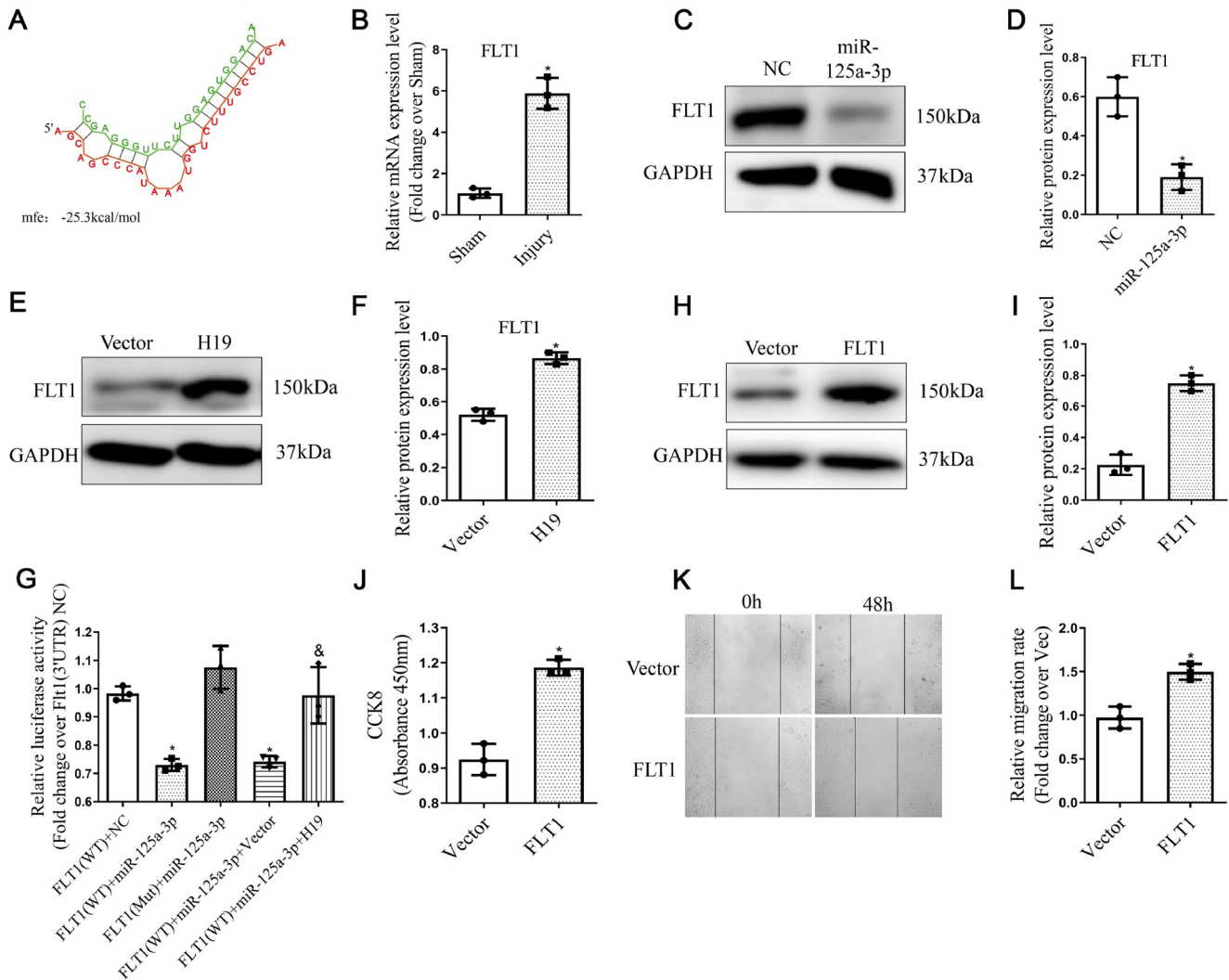


Figure 5. lncRNA H19 promoted VSMC proliferation and migration by suppressing miR-125a-3p to induce FLT1 expression (A) Bioinformatics analysis of the binding sites between miR-125a-3p and FLT1. (B) Relative mRNA expression of FLT1 in arteries collected from mouse sham carotid arteries and injured carotid arteries. $*P < 0.05$ versus the sham group ($n = 5$). (C) Western blot analysis of FLT1 in human VSMCs treated with NC or miR-125a-3p mimics. (D) Quantitative analysis of the FLT1 protein level. $*P < 0.05$ versus the NC group ($n = 3$). (E) Western blot analysis of FLT1 in human VSMCs. (F) Quantitative analysis of the FLT1 protein level in (D). $*P < 0.05$ versus the vector group ($n = 3$). (G) Dual-luciferase reporter assay of human 293T cells treated with NC mimics, miR-125a-3p mimics, and the FLT1-WT or FLT1-Mut luciferase reporters. $*P < 0.05$ versus the FLT1 (WT) + NC group; $^{\&}P < 0.05$ versus the FLT1(WT) + miR-125a-3p + vector group ($n = 3$). (H) Western blot analysis of FLT1 in human VSMCs infected with vector or FLT1 lentivirus. (I) Quantitative analysis of the FLT1 protein level. $*P < 0.05$ versus the vector group ($n = 3$). (J) The proliferation of human VSMCs infected with vector or FLT1 lentivirus was measured by a CCK-8 assay. $*P < 0.05$ versus the vector group ($n = 5$). (K) The migration of human VSMCs infected with vector or FLT1 lentivirus. Magnification: $100\times$. (L) Quantitative analysis of the migration distance. $*P < 0.05$ versus vector ($n = 3$).

examined the potential interaction between lncRNA H19 and miR-125a-3p via RNA immunoprecipitation and luciferase reporter assays. As expected, we observed that lncRNA H19 directly bound to miR-125a-3p and downregulated its expression. Moreover, the miR-125a-3p mimic significantly suppressed the increase in VSMC proliferation and migration induced by lncRNA H19.

To identify target genes of the lncRNA H19/miR-125a-3p axis, we applied bioinformatics analysis and found that FLT1 has a potential binding site for miR-125a-3p. FLT1 is reportedly a pivotal protein involved in cell proliferation and migration. For example, miR-145-5p promotes trophoblast cell growth and invasion by targeting FLT1 [31,32]. However, the role of FLT1 in VSMCs is still largely

unknown. Our data showed that FLT1 was a direct target of miR-125a-3p in VSMCs and that overexpression of FLT1 dramatically enhanced the proliferation and migration of VSMCs, indicating that the miR-125a-3p/FLT1 axis may mediate the effects of lncRNA H19 on the proliferation and migration of VSMCs. However, the role of FLT1 in VSMCs needs to be addressed in future studies. To extend the *in vitro* findings to an *in vivo* model, we determined the effect of lncRNA H19 overexpression on neointima formation in an animal model of vascular injury. Our results showed that upregulating lncRNA H19 significantly decreased miR-125a-3p expression and increased FLT1 expression. Moreover, the overexpression of lncRNA H19 markedly promoted neointima formation after vascular

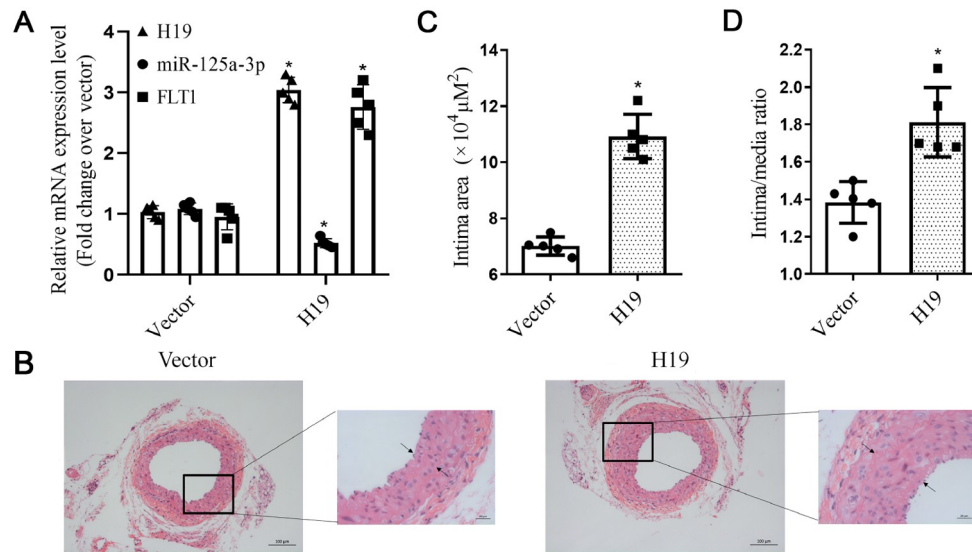


Figure 6. lncRNA H19 promoted neointima formation after vascular injury *in vivo*. (A) Relative expression of lncRNA H19, miR-125a-3p and FLT1 in mouse carotid arteries incubated with vector lentivirus or lncRNA H19 lentivirus. $*P < 0.05$ versus the vector group ($n = 5$). (B) H&E staining of carotid arteries incubated with vector lentivirus and lncRNA H19 lentivirus after vascular injury. The ligated artery was incubated with $100 \mu\text{l}$ of Pluronic F127 gel (25% w/v dissolved in 0.9% saline) containing vector lentivirus or lncRNA H19 overexpression lentivirus ($1 \times 10^9 \text{ Tu/mL}$) for 20 min. Two weeks after ligation, the carotid arteries were excised and analyzed. Arrows indicate the neointima area. Scale bar = $100 \mu\text{m}/20 \mu\text{m}$. (C) Quantitation of the intima/media ratio. $*P < 0.05$ versus the vector group ($n = 5$). (D) Quantitation of the neointima area. $*P < 0.05$ versus the vector group ($n = 5$). All data are presented as the mean $\pm$ SD (unpaired, two-tailed Student's *t* test for C and D; one-way ANOVA with Tukey's post hoc test for A).

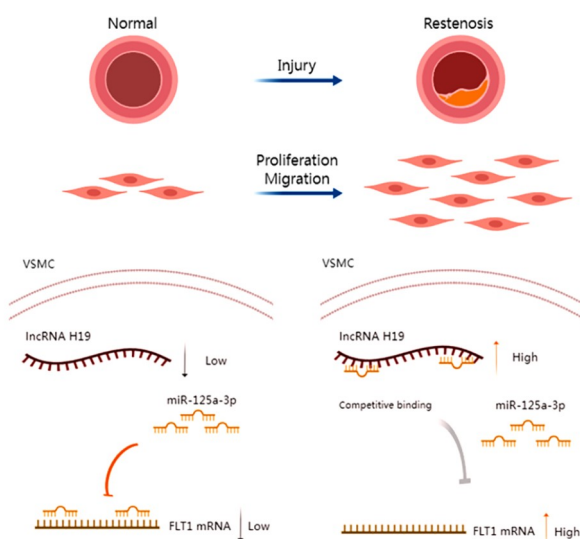


Figure 7. Schematic diagram summarizing the role of the lncRNA H19/miR-125a-3p/FLT1 axis in neointima formation. lncRNA H19 overexpression significantly promoted neointima formation *in vivo* and VSMC proliferation and migration *in vitro*. Mechanistically, lncRNA H19 acted as a ceRNA of miR-125a-3p and facilitated the expression of the miR-125a-3p target FLT1 in VSMCs.

injury *in vivo*.

In conclusion, lncRNA H19 stimulates VSMC proliferation and migration by acting as a ceRNA of miR-125a-3p. We speculate that the lncRNA H19/miR-125a-3p/FLT1 axis could serve as a potential target for the treatment of restenosis after angioplasty or stenting (Figure 7). Moreover, our current study identified a new therapeutic target for diseases associated with abnormal VSMC proliferation

and migration, such as hypertension, postangioplasty restenosis, and aneurysm, for clinical treatment.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

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Conflict of Interest

The author declares that there is no conflict of interest.

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