miR-541-3p inhibits the viability and migration of vascular smooth muscle cells via targeting STIM1

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Abstract. The transformation of vascular smooth muscle cells (VSMCs) into the proliferative migratory phenotype in the plaque area contributes to stable plaque formation and facilitates the pathogenesis of atherosclerosis. Stromal interaction molecule 1 (STIM1) has been identified to promote the proliferation of VSMCs, suggesting that STIM1 may be a potent target for the prevention and treatment of atherosclerosis. Bioinformatics analysis has previously predicted STIM1 as a target of microRNA (miR)-541-3p. The present study aimed to determine the effect of the miR-541-3p/STIM1 axis on the progression of atherosclerosis *in vitro*. Oxidized low-density lipoprotein (ox-LDL)-treated VSMCs were used as an *in vitro* atherosclerosis model. Cell Counting Kit-8 and Transwell migration assays were used to analyze cell viability and migration, respectively. Reverse transcription-quantitative PCR and western blotting were applied to measure mRNA and protein expression levels, respectively. The association between miR-541-3p and STIM1 was detected using a dual luciferase gene reporter assay. The results of the present study revealed that ox-LDL treatment significantly downregulated miR-541-3p expression levels and upregulated STIM1 expression levels in VSMCs. In addition, ox-LDL stimulation enhanced cell viability and migration. The overexpression of miR-541-3p significantly reversed the ox-LDL-mediated increase in cell viability and migration, whereas the knockdown of miR-541-3p expression enhanced the ox-LDL-mediated effects. STIM1 was confirmed to be a target gene of miR-541-3p in VSMCs. The knockdown of STIM1 significantly impaired the stimulatory effects of miR-541-3p knockdown on cell viability and migration. In conclusion, the findings of the present study suggested that miR-541-3p may efficiently repress VSMC viability and migration by targeting STIM1 under the treatment of ox-LDL. These results indicated that the miR-541-3p/STIM1 axis may represent a potent target to modulate VSMC viability and migration.

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Introduction

Atherosclerosis is a chronic progressive inflammatory disease and is the leading cause of mortality worldwide due to the progression to myocardial infarction and stroke, which are major clinical consequences (1,2). It is widely accepted that the transformation of vascular smooth muscle cells (VSMCs) from a quiescent contractile phenotype to a proliferative migratory phenotype facilitates stable plaque formation, contributing to the pathogenesis of atherosclerosis (3). However, the molecular mechanisms underlying the proliferation and migration of VSMCs in the pathogenesis of atherosclerosis remain to be fully understood.

MicroRNAs (miRNAs/miRs) are evolutionarily conserved, non-coding small RNAs that can post-transcriptionally modulate gene expression by binding to the 3'-untranslated region (UTR) of target mRNA (4). miRNAs have been shown to play pivotal roles in tumorigenesis, biological metabolism and immune inflammation by modulating cell proliferation, apoptosis, migration, invasion and differentiation (5-8). Increasing evidence has revealed that miRNAs may be closely associated with the pathogenesis of atherosclerosis through the modulation of VSMC proliferation and migration (9-12). Therefore, targeting miRNAs may represent a potentially effective method for the treatment of atherosclerosis.

Sustained Ca^{2+} influx mediated by store-operated channels (SOCs) is triggered in response to Ca^{2+} depletion by the endoplasmic reticulum (ER), and has been discovered to serve an important role in numerous cellular functions, such as cell proliferation and apoptosis (13). Stromal interaction molecule 1 (STIM1) has been reported to modulate SOCs by functioning as an ER Ca^{2+} sensor and has also been reported to modulate

the proliferation of VSMCs (14,15). Using bioinformatics technology, TargetScan (http://www.targetscan.org/vert_71) and miRDB (http://www.mirdb.org/index.html), STIM1 was predicted as a target gene of miR-541-3p in the present study. Thus, it was hypothesized that miR-541-3p may be involved in the pathogenesis of atherosclerosis by targeting STIM1.

The present study aimed to determine the role of the miR-541-3p/STIM1 axis in the biological behaviors of VSMCs. Oxidized low-density lipoprotein (ox-LDL)-treated VSMCs were used as an *in vitro* model of atherosclerosis, and VSMC viability and migration were detected to investigate the pathogenesis of atherosclerosis *in vitro*.

Materials and methods

Cell culture and treatment. Human VSMCs (cat. no. CRL-1999™) were purchased from the American Type Culture Collection and cultured in F-12K medium (Gibco; Thermo Fisher Scientific, Inc.), supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.). The cells were maintained at 37˚C in a humidified incubator with 5% CO_2.

To construct the *in vitro* atherosclerosis model, VSMCs were treated with 100 mg/l ox-LDL (cat. no. H7950; Beijing Solarbio Science & Technology Co., Ltd.) for 24 or 48 h at 37˚C. The untreated VSMCs served as a control.

Cell transfection. miR-541-3p mimic and inhibitor (used to overexpress and knockdown miR-541-3p expression in VSMCs, respectively), small interfering RNA (siRNA/si) targeting STIM1 (si-STIM1) and the negative controls (NCs), mimic-NC, inhibitor-NC and si-NC (non-targeting sequences) were all synthesized by Shanghai GenePharma Co., Ltd. The mimic (150 nM), inhibitor (100 nM), si-STIM1 (50 nM) and the same amount of control vectors were transfected into $1x10^6$ cells using Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature according to the manufacturer's protocol. The transfection efficiency was detected by RT-qPCR and western blotting 48 h post-transfection. The sequences were as follows: Mimic, 5'-TGGTGGGCACAGAATCTGGACT-3'; mimic-NC, 5'-UUCUCCGAACGUGUCACGUTT-3'; inhibitor, 5'-AGTCCAGATTCTGTGCCCACCA-3'; inhibitor-NC, 5'-ACAGGAUUGAGGGGGGGCCCU-3'; si-STIM1, 5'-GAGGTGCAATATTACAACATCAAGA-3'; si-NC, 5'-GAGAACGTTATAACACTACATGAGA-3'.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from VSMCs using the RNApure Tissue & Cell kit (CoWin Biosciences), according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed into cDNA for miRNA and mRNA detection using stem-loop primers and random primers using EasyScript® First-Strand cDNA Synthesis SuperMix (Beijing Transgen Biotech Co., Ltd.) according to the manufacturer's protocol, respectively. qPCR was subsequently performed using a TaqMan Universal Master mix II kit (Thermo Fisher Scientific, Inc.) on a Bio-Rad detection system (Bio-Rad Laboratories, Inc.). GAPDH and U6 served as the internal references for the normalization of mRNA and miRNA expression, respectively. The relative expression was calculated using the $2^{-\Delta\Delta Cq}$ method (16). PCR thermocycling conditions were as follows: Initial denaturation at 95˚C for 30 sec, followed by 39 cycles at 95˚C for 15 sec and 60˚C for 30 sec, and a final step at 72˚C for 5 min. The following primer pairs were used: Stem-loop primer (used for RT of miR-541-3p), 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGTCCA-3'; miR-541-3p forward, 5'-TGGTGGGCACAGAATC-3' and reverse, 5'-GTGCAGGGTCCGAGGT-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; STIM1 forward, 5'-GTTCTGAAGGCTACGGGACC-3' and reverse, 5'-GTCAGAAGGGGGTGTGTCAG-3'; GAPDH forward, 5'-CCACTAGGCGCTCACTGTTCTC-3' and reverse, 5'-ACTCCGACCTTCACCTTCCC-3'.

Western blotting. Total protein was extracted from VSMCs using RIPA lysis buffer (Sangon Biotech Co., Ltd.) supplemented with protease inhibitor (Beijing Solarbio Science & Technology Co., Ltd.). Total protein was quantified using a BCA protein kit (Bio-Rad Laboratories, Inc.) and 30 μg protein from each sample was loaded and separated via SDS-PAGE on 10% gels. The separated proteins were subsequently transferred onto polyvinylidene difluoride membranes (EMD Millipore) and blocked with 5% non-fat milk for 1 h at room temperature. The membranes were then incubated with the following primary antibodies overnight at 4˚C: Anti-STIM1 (cat. no. 4916; Cell Signaling Technology, Inc.; 1:3,000 dilution), anti-VEGF (cat. no. 2463; Cell Signaling Technology, Inc. 1:3,000 dilution), anti-matrix metalloproteinase (MMP)9 (cat. no. 13667; Cell Signaling Technology, Inc.; 1:3,000 dilution), anti-phosphorylated (p)-PI3K p85 (cat. no. 17366; Cell Signaling Technology, Inc.; 1:2,000 dilution), anti-PI3K p85 (cat. no. 4292; Cell Signaling Technology, Inc.; 1:3,000 dilution), anti-p-AKT1 (cat. no. 9018; Cell Signaling Technology, Inc.; 1:2,000 dilution), anti-AKT1 (cat. no. 2938; Cell Signaling Technology, Inc.; 1:3,000 dilution) and anti-GAPDH (cat. no. 2118; Cell Signaling Technology, Inc.; 1:2,000 dilution). Following the primary antibody incubation, the membranes were incubated with the goat anti-rabbit IgG HRP-linked secondary antibodies (cat. no. sc-2004; Santa Cruz Biotechnology, Inc.; 1:5,000 dilution) for 1 h at room temperature. Protein expression levels were determined using a western blotting imaging and quantitative system (Gel Doc XR+; Bio-Rad Laboratories, Inc.) following incubation with the ECL western blotting substrate (EMD Millipore). Densitometric analysis was performed using ImageJ software (version 1.6.0; National Institutes of Health) after background subtraction, with GAPDH expression levels acting as the internal reference control.

Dual luciferase gene reporter assay. The putative binding sites between miR-541-3p and STIM1 were predicted using TargetScan (http://www.targetscan.org/vert_71) and miRDB (http://www.mirdb.org/index.html). The wild-type (WT) and mutant (MUT) type of the 3'-UTR of STIM1, which were cloned into the pGL3 vector (Promega Corporation), were constructed by Shanghai GenePharma Co., Ltd.; the resulting plasmids were denoted as STIM1-WT or STIM1-MUT reporter plasmids, respectively. VSMCs ($1x10^5$) were co-transfected with WT (0.2 μg) or MUT (0.2 μg) and miR-541-3p mimic (150 nM) or mimic-NC (150 nM), as well as the *Renilla* luciferase vector (0.2 μg; control) using Lipofectamine 2000 transfection reagent. A total of 48 h post-transfection, the relative luciferase activity

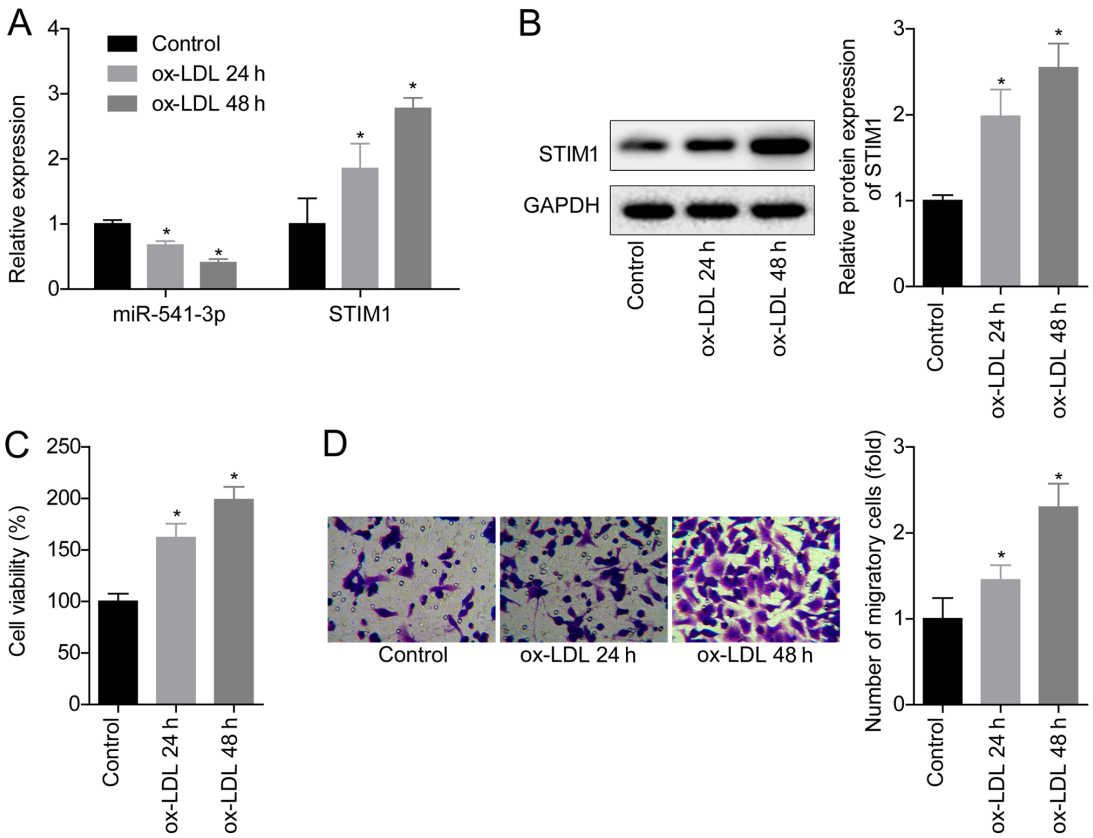

Figure 1. Ox-LDL treatment downregulates miR-541-3p expression levels and upregulates STIM1 expression levels in VSMCs. VSMCs were pretreated with 100 mg/l ox-LDL for 24 or 48 h. (A) mRNA expression levels of miR-541-3p and STIM1 were analyzed using reverse transcription-quantitative PCR. (B) Protein expression levels of STIM1 were determined using western blotting. (C) Cell Counting Kit-8 assay was used to analyze cell viability. (D) Cell migration was analyzed using a Transwell assay. Magnification, x200. n=3; *P<0.05 vs. control (one-way ANOVA and Bonferroni post hoc test). Ox-LDL, oxidized low-density lipoprotein; miR, microRNA; STIM1, stromal interaction molecule 1; VSMCs, vascular smooth muscle cells.

normalized to *Renill*a luciferase activity was measured using a Dual Luciferase Reporter assay system (Promega Corporation) according to the manufacturer's protocol.

Cell Counting Kit-8 (CCK-8) assay. VSMCs were seeded into 96-well plates at a density of $3x10^3$ cells/well and cultured at 37˚C overnight, followed by transfection with different vectors as aforementioned. After incubation at 37˚C for 24 or 48 h, cell culture medium was replaced with 10 μl CCK-8 reagent (Beyotime Institute of Biotechnology) and 90 μl fresh medium containing 10% FBS, and incubated at 37˚C for a further 4 h. The optical density (OD) value was measured at a wavelength of 450 nm using a microplate reader (model 680; Bio-Rad Laboratories, Inc.). Cell viability (%) = OD (treatment group)/OD (control group) x100.

Transwell migration assay. Transwell chambers (BD Biosciences) were used to determine the cell migration ability. Briefly, ~$3x10^5$ VSMCs were resuspended in serum-free medium and seeded into the upper chamber of the Transwell plates. The lower chambers were filled with 600 μl medium supplemented with 15% FBS. After incubation at 37˚C for 48 h, the cells remaining on the top of the membranes were removed using cotton buds, while cells on the lower membrane were fixed with -20˚ C-precooled absolute methanol for 15 min and stained with 0.2% crystal violet (Beijing Solarbio Science & Technology Co., Ltd.) for 5 min all at room temperature. The stained cells were subsequently counted under a light microscope (magnification, x200) to assess cell migration.

Statistical analysis. Data were presented as the mean ± SD. Statistical analysis was performed using SPSS 21.0 software (IBM Corp.). Each experiment was performed in triplicate. Statistical differences between two groups and multiple groups were performed using an unpaired Student's t-test or one-way ANOVA followed by a Bonferroni's post hoc test, respectively. P<0.05 was considered to indicate a statistically significant difference.

Results

Ox-LDL treatment downregulates the expression levels of miR-541-3p and upregulates STIM1 expression levels. To determine the role of the miR-541-3p/STIM1 axis in the pathogenesis of atherosclerosis, the expression levels of miR-541-3p and STIM1 in VSMCs following the treatment with ox-LDL were analyzed. The results demonstrated that ox-LDL treatment significantly downregulated miR-541-3p expression levels and significantly upregulated STIM1 expression levels compared with the untreated VSMCs in the control group in a time-dependent manner (Fig. 1A and B). VSMC viability was significantly increased following treatment with 100 mg/l ox-LDL for 24 and 48 h compared with the control group (Fig. 1C), as was the cell migratory ability (Fig. 1D). These

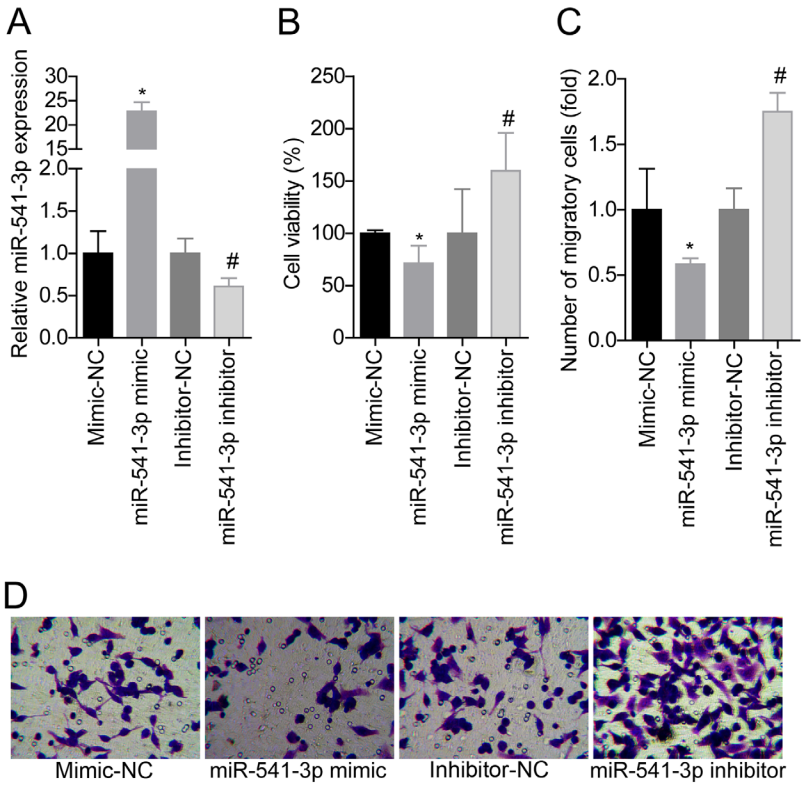

Figure 2. Effects of miR-541-3p on VSMC viability and migration. VSMCs were pretreated with 100 mg/l ox-LDL for 24 h and then transfected with a miR-541-3p inhibitor, inhibitor-NC, miR-541-3p mimic or mimic-NC. (A) Reverse transcription-quantitative PCR was used to analyze the expression levels of miR-541-3p. (B) Cell Counting Kit-8 assay was used to determine cell viability. (C and D) Cell migration was analyzed using a Transwell assay. Magnification, x200. n=3; *P<0.05 vs. mimic-NC; $^{\#}$P<0.05 vs. inhibitor-NC (one-way ANOVA and Bonferroni post hoc test). miR, microRNA; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein; NC, negative control.

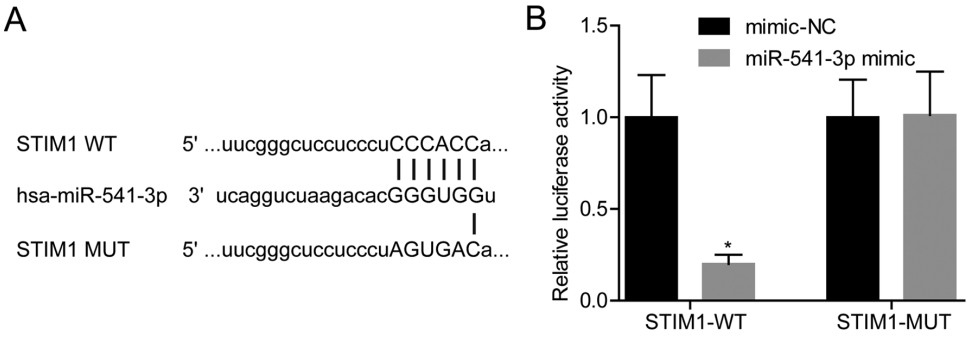

Figure 3. miR-541-3p targets STIM1 in vascular smooth muscle cells. (A) Putative binding sites between miR-541-3p and the 3'-untranslated region of STIM1 were identified. (B) Dual luciferase reporter assay was used to determine the effect of miR-541-3p overexpression on the relative luciferase activity of STIM1. n=3; *P<0.05 vs. mimic-NC + STIM1-WT (unpaired Student's t-test). miR, microRNA; STIM1, stromal interaction molecule 1; WT, wild-type; MUT, mutant.

results indicated that ox-LDL treatment may downregulate miR-541-3p expression and upregulate STIM1 expression, which may be involved in process of atherosclerosis.

Overexpression of miR-541-3p inhibits the viability and migration of VSMCs. The effects of miR-541-3p on cell viability and migration in ox-LDL (100 mg/l; 24 h)-treated VSMCs were analyzed using gain- and loss-of-function assays. Since ox-LDL treatment for 24 h caused significant differences in cell viability and migration, this timepoint was assessed in subsequent experiments. miR-541-3p expression levels were significantly downregulated following the transfection of VSMCs with the miR-541-3p inhibitor, and significantly upregulated following transfection with the miR-541-3p mimic compared with the respective NCs (Fig. 2A). The cell viability (Fig. 2B) and migratory abilities (Fig. 2C and D) were significantly weakened following the overexpression of miR-541-3p in VSMCs compared with the mimic-NC-transfected VSMCs, whereas knockdown of miR-541-3p expression exacerbated ox-LDL-induced stimulation of cell viability and migration compared with the inhibitor-NC-transfected VSMCs (Fig. 2B-D). These results suggested that miR-541-3p may suppress the ox-LDL-induced induction of cell viability and migration in VSMCs.

miR-541-3p targets STIM1 in VSMCs. As shown in Fig. 3A, STIM1 was a putative target of miR-541-3p, which was

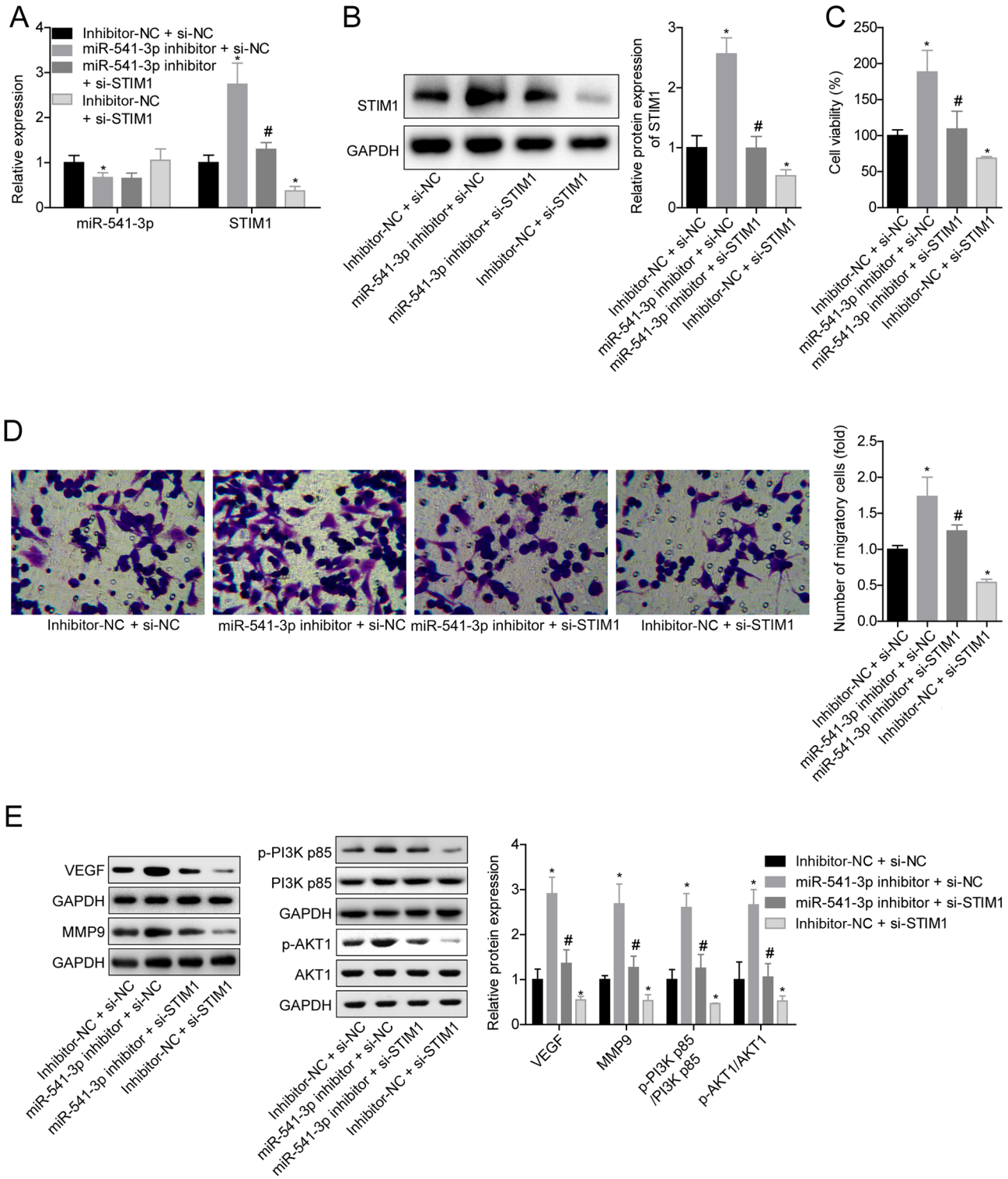

Figure 4. Effects of the miR-541-3p/STIM1 axis on cell viability and migration, and the activation of PI3K/AKT signaling in VSMCs. VSMCs were pretreated with 100 mg/l ox-LDL for 24 h, followed by transfection with inhibitor-NC + si-NC, miR-541-3p inhibitor + si-NC, miR-541-3p inhibitor + si-STIM1 or inhibitor-NC + si-STIM1. (A) Expression levels of miR-541-3p and STIM1 were analyzed using reverse transcription-quantitative PCR. (B) Protein expression levels of STIM1 were analyzed using western blotting. (C) Cell Counting Kit-8 assay was used to analyze cell viability. (D) Transwell assay was used to determine cell migration. Magnification, x200. (E) Expression levels of VEGF, MMP9, p-PI3K p85, PI3K p85, p-AKT1 and AKT1 were analyzed using western blotting. n=3; *P<0.05 vs. inhibitor-NC + si-NC; $^{\#}$P<0.05 vs. miR-541-3p inhibitor + si-NC (one-way ANOVA and Bonferroni post hoc test). miR, microRNA; STIM1, stromal interaction molecular 1; VSMCs, vascular smooth muscle cells; ox-LDL, oxidized low-density lipoprotein; NC, negative control; si, small interfering RNA; p-, phosphorylated; MMP9, matrix metalloproteinase 9.

confirmed by the dual luciferase gene reporter assay using STIM1-WT or STIM1-MUT reporter plasmids (Fig. 3B). The results revealed that overexpression of miR-541-3p in VSMCs transfected with STIM1-WT induced a significant reduction in luciferase activity; however, the mutation in the binding site between miR-541-3p and the 3'UTR of STIM1 abrogated this

effect (Fig. 3B). These results suggested that miR-541-3p may target STIM1 in VSMCs.

Knockdown of miR-541-3p promotes ox-LDL-induced VSMC viability and migration by targeting STIM1. CCK-8 and Transwell assays were subsequently performed to investigate the role of the miR-541-3p/STIM1 axis in ox-LDL-induced VSMC viability and migration. The transfection with si-STIM1 significantly downregulated STIM1 expression at both the mRNA and protein levels compared with the si-NC group (Fig. S1A and B). In addition, the expression levels of STIM1 were significantly upregulated in VSMCs in the miR-541-3p inhibitor + si-NC group as compared with in the inhibitor-NC + si-NC group, while the expression levels were significantly downregulated in cells transfected with si-STIM1 in the inhibitor-NC + si-STIM1 group (Fig. 4A and B). Knockdown of STIM1 in the miR-541-3p inhibitor + si-STIM1 group impaired the miR-541-3p inhibitor-induced stimulatory effect on cell viability and migration in ox-LDL (100 mg/l; 24 h)-treated VSMCs as compared with the inhibitor-NC + si-NC group (Fig. 4C and D), indicating that knockdown of miR-541-3p may promote ox-LDL-induced VSMC viability and migration by targeting STIM1.

In addition, the knockdown of miR-541-3p in the miR-541-3p inhibitor + si-NC group significantly upregulated the expression levels of VEGF and MMP9, and the p-AKT1:AKT1 and p-PI3K:PI3K ratios as compared with the inhibitor-NC + si-NC group. However, transfection with si-STIM1 in the inhibitor-NC + si-STIM1 group induced the opposite results and rescued the effect of the miR-541-3p inhibitor (miR-541-3p inhibitor + si-STIM1 group vs. miR-541-3p inhibitor + si-NC group) (Fig. 4E). These results suggested that the miR-541-3p/STIM1 axis may modulate VSMC viability and migration via PI3K/AKT signaling.

Discussion

The enhanced proliferation of VSMCs has been identified as the main mechanism of aberrant neointima formation in vascular diseases (17). During atherogenesis, VSMCs respond to several factors, including thrombin, platelet derived growth factor-BB, IFN-γ, endothelin-1 and IL-1, resulting in the migration of VSMCs to the intima (18,19). The migration of VSMCs leads to plaque formation and also facilitates the atherosclerosis hardening process (20). Thus, inhibition of VSMC proliferation and migration is a potential strategy for the prevention or treatment of atherosclerosis (21). The present study aimed to determine the role of the miR-541-3p/STIM1 axis in the viability and migration of VSMCs. The results illustrated that miR-541-3p suppressed ox-LDL-mediated VSMC viability and migration by targeting STIM1.

To date, numerous miRNAs have been reported to play an important role in the pathogenesis of atherosclerosis by modulating VSMC proliferation and migration (22). For example, miR-146a expression was revealed to be upregulated in proliferating VSMCs, and knockdown of miR-146a expression weakened the proliferative and migratory capacities of VSMCs *in vitro* (23). In addition, miR-92a expression was upregulated in the atherosclerotic plaques of mice and the overexpression of miR-92a significantly increased the proliferation of VSMCs (24). Xu *et al* (25) also reported that miR-647 expression was upregulated in the serum samples of patients with atherosclerosis and ox-LDL-treated VSMCs, and the upregulation of miR-647 expression levels promoted the proliferation and migration of ox-LDL-treated VSMCs via targeting PTEN. miR-541-3p expression levels were also shown to be downregulated in non-small cell lung cancer (NSCLC) tissues and plasma, and the overexpression subsequently suppressed NSCLC cell growth and metastasis (26). To the best of our knowledge, the present study was the first to demonstrate that miR-541-3p expression levels were downregulated in ox-LDL-treated VSMCs, and that the overexpression of miR-541-3p significantly suppressed ox-LDL-induced increases in VSMC viability and migration.

Using TargetScan and miRDB, the current study predicted that miR-541-5p was a regulator of STIM1, which has been previously identified to promote VSMC proliferation (14,15). Subsequently, dual luciferase gene reporter assays were used to verify that STIM1 was a target gene of miR-541-3p in VSMCs. Further *in vitro* experiments revealed that the genetic knockdown of STIM1 significantly weakened the miR-541-3p knockdown-induced stimulation of VSMC viability and migration, indicating that miR-541-3p may inhibit ox-LDL-induced VSMC viability and migration by targeting STIM1. miR-541-5p was found to negatively regulate STIM1 expression by binding to the 3'-UTR of STIM1 mRNA, leading to the inhibition of VSMC viability. The present results showed that STIM1 may serve a role in regulating cell viability, which was consistent with previous studies (27-30), which reported that the dysregulation of STIM1 caused a deregulation in cell viability.

The PI3K/AKT signaling pathway, one of the most important intracellular pathways in the body, is involved in a variety of cellular processes, including cell proliferation, survival, differentiation and migration (31). Notably, PI3K/AKT signaling was also discovered to play a crucial role in the pathogenesis of atherosclerosis (32). It has previously been reported that the selective inhibition of PI3K/AKT signaling could potently suppress the progression of atherosclerosis (33,34), whereas the activation of PI3K/AKT signaling enhanced the proliferation, survival and migration of VSMCs and human umbilical vein endothelial cells (35). These findings indicated that PI3K/AKT signaling may be an important target for the treatment of atherosclerosis. Therefore, the present study investigated the effect of the miR-541-3p/STIM1 axis on the activation of PI3K/AKT signaling. The results revealed that the knockdown of miR-541-3p significantly increased the phosphorylation levels of PI3K and AKT; however, this trend was abolished following the silencing of STIM1 in ox-LDL-treated VSMCs. Consistent with these findings, a previous study reported that the knockdown of STIM1 inactivated PI3K/AKT signaling in human prostate cancer cells (36). These aforementioned results suggested that PI3K/AKT signaling may serve a role in the miR-541-5p/STIM1 axis-mediated inhibition of VSMC viability and migration.

There are two main limitations of the present study. Firstly, the specific role of the PI3K/AKT signaling pathway in the miR-541-5p/STIM1 axis-mediated inhibition of VSMC viability and migration was not clarified. Secondly, the role of the miR-541-3p/STIM1 axis was only investigated *in vitro*.

Thus, *in vivo* experiments should be performed in future studies to determine the role of the miR-541-3p/STIM1 axis in the pathogenesis of atherosclerosis.

In conclusion, the findings of the present study suggested that miR-541-3p may efficiently suppress ox-LDL-induced increases in VSMC viability and migration by targeting STIM1, which may be associated with the suppression of PI3K/AKT signaling. These results indicated that the miR-541-3p/STIM1 axis may represent a potential target to modulate VSMC viability and migration.

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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

ZL, DY, CZ and YX designed the study, analyzed the data and interpreted the results. ZL, DY, YX and HH performed experiments. ZL, DY, CZ and YX wrote the manuscript and prepared the figures. ZF and XL analyzed data, and reviewed and edited the manuscript. XL coordinated and supervised the study. ZL and XL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

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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