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LncRNA ENST00113 promotes proliferation, survival, and migration by activating PI3K/Akt/mTOR signaling pathway in atherosclerosis

Xinliang Yao, MD, Chengyun Yan, MD, Lei Zhang, MD, Yanming Li, MD, Qilin Wan, MD, PhD*

Abstract

Background: Atherosclerosis is one of the most common cardiovascular disorders. The dysfunction of vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) are 2 key factors in the formation of atherosclerosis. This study aims to find strategies to prevent VSMCs and ECs dysfunction for the treatment of atherosclerosis.

Methods: We investigated the expression patterns of long noncoding RNAs (lncRNAs) in 2 pairs of serum samples from both atherosclerosis patients and healthy volunteers through microarray analysis. Then we selected the most up-regulated lncRNA ENAST00113 (lnc00113) to further verify its roles in atherosclerosis. VSMCs, and human umbilical vein endothelial cells (HUVECs) transfected with small interfering RNA (siRNAs) (si-00113-1, si-00113-1) and a negative control (si-NC) were cultured. MTT assay, Caspase 3 enzyme-linked immunosorbent assay (ELISA) assay, and wound healing assay were performed to evaluate whether lnc00113 had an effect on proliferation, apoptosis, and migration ability. Further, the correlation between lnc00113 and PI3K/Akt/mTOR signaling pathway was explored.

Results: Microarray results indicated that 243 lncRNAs were up-regulated and 187 lncRNAs were down-regulated. Therefore, we chose the most up-regulated lncRNA ENST (lnc00113) to further explore its roles in atherosclerosis. Real-time polymerase chain reaction (RT-qPCR) results showed that the expression of lnc00113 was highly increased in atherosclerosis patients. In vitro experiment demonstrated that lnc00113 down-regulation significantly suppressed VSMCs and HUVECs proliferation, survival, and migration. Furthermore, we found that the protein expressions of phosphorylated-PI3K (p-PI3K), phosphorylated-Akt (p-Akt), phosphorylated-mTOR (p-mTOR), and bcl-2 in HUVECs cells transfected with si-00113-1 or si-00113-2 were dramatically decreased compared with si-NC-transfected cells and control cells. However, the total- PI3K (t-PI3K), total-Akt (t-Akt), and total-mTOR (t-mTOR) protein expressions were not changed, indicating that lnc00113 could activate PI3K/Akt/mTOR signaling pathway in atherosclerosis.

Conclusions: This finding identified an important role of lnc00113 in VSMCs and HUVECs that promotes cell proliferation, survival, and migration by activating PI3K/Akt/mTOR signaling pathway, which could probably serve as a promising therapeutic target for atherosclerosis.

Abbreviations: BCA = bicinchoninic acid assay, Bvht = braveheart, Ct = cycle threshold, ECL = electrochemiluminescence, ECs = endothelial cells, HOXC6 = homeobox C6, HOXC-AS1 = HOXC cluster antisense RNA 1, HRP = horseradish peroxidase, HUVECs = human umbilical vein endothelial cellslinc, lncRNAs = long noncoding RNAs, miRNAs = microRNAs, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, ncRNAs = noncoding RNAs, OD = optical density, oxLDL = low-density lipoprotein, p-Akt = phosphorylated-Akt, piRNAs = piwi-interacting RNA, p-mTOR = phosphorylated-mTOR, p-PI3K = phosphorylated-PI3K, RNAs = long intergenic noncoding RNAs, RT-qPCR = real-time polymerase chain reaction, SD = standard deviation, SDS = sodium dodecyl sulfate, SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis, siRNAs = small interfering RNA, TBST = tris-HCl buffer solution-Tween, TNA-α = tumor necrosis factor α, VSMCs = vascular smooth muscle cells.

Keywords: atherosclerosis, endothelial cell (EC), LncRNA ENST00113 (lnc00113), PI3K/Akt/mTOR signaling pathway, vascular smooth muscle cell (VSMC)

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Department of Cardiology, Huaihe Hospital of Henan University, Kaifeng, Henan, China.

** Correspondence: Qilin Wan, Department of Cardiology, Huaihe Hospital of Henan University, No. 8 Baobei Road, Gulou District, Kaifeng 475000, Henan Province, China (e-mail: wanqilin_hn@163.com).*

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1. Introduction

As one of the major causes of mortality and morbidity, atherosclerosis is one of the most common cardiovascular disorders at present, even though both basic and clinical researches have made great progress on it.[1] Atherosclerosis is a clinical symptom that causes narrowing of the interior of an artery due to plaque accumulation. Several serious risk factors, such as excessive drinking, smoking, eating disorder, lack of exercises, and obesity, may promote the development and progression of cardiovascular disease and premature death. Atherosclerosis can be activated by dysfunction of endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), proinflammatory cytokines produced by macrophages such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α), etc.[2,3] ECs and VSMCs dysfunction include inappropriate cell apoptosis, proliferation, migration, and abnormal expression of adhesion molecule proteins. Endothelial dysfunction is considered an early sign of atherosclerosis before angiographic or ultrasound evidence of atherosclerotic plaques.[4] VSMCs are activated and regain their hyperproliferative properties under certain pathological conditions by thickening and hardening the arterial wall. Thus, VSMCs plays a pivotal role in the development and eventual rupture of atherosclerotic plaques in this process,[5] and finding strategies to prevent dysfunction of ECs and VSMCs is important for the treatment of atherosclerosis.

Over 90% of the human genome is transcribed, however protein-coding genes only account for less than 2% of the human genome.[6–9] The majority of transcripts are noncoding RNAs (ncRNAs) including the well-known microRNAs (miRNAs), the newly studied snRNAs, piwi-interacting RNA (piRNAs), small interfering RNA (siRNAs), as well as long noncoding RNAs (lncRNAs).[10] lncRNAs is also known as long intergenic noncoding RNAs (lincRNAs) with transcripts longer than 200 nucleotides.[11,12] Once lncRNAs were thought to have no function, but accumulating evidence has demonstrated that lncRNA could take part in various biological and pathological processes like carcinogenesis and chronic diseases.[13] Therefore, understanding the role of lncRNAs in atherosclerosis may provide novel and effective therapeutic methods for atherosclerosis.

Recent study suggests that several noncoding RNAs are involved the development and progression in atherosclerosis. Investigators described the mechanistic role of miRNAs in the regulation of the pathophysiology of important cardiovascular processes including atherosclerosis, angiogenesis, and in-stent restenosis after angioplasty.[13] The specific role of miR-143/145 and miR-126, major regulators of ECs and VSMCs function, has been further investigated. Investigators found a prototype of heart-related mouse lncRNA braveheart (Bvht) and Bvht essential for the maintenance of cardiomyocyte fate.[14] According to 1 study, lncRNA-HOXC cluster antisense RNA 1 (HOXC-AS1) is significantly down-regulated expression in atherosclerosis. The results show that lncRNA-HOXC-AS1 can suppress oxidized low-density lipoprotein-induced cholesterol accumulation in THP-1 macrophages by promoting homeobox C6 (HOXC6) expression. This indicates that lncRNA HOXC-AS1 can be perceived as a method of therapy for atherosclerosis.[15] Previous studies have shown that lncRNAs can be important regulators of cell apoptosis, proliferation, and differentiation of ECs and VSMCs.[16–18] Although previous studies involved some noncoding RNAs, the exact mechanisms of atherosclerosis and lncRNAs were largely unknown until now. In the present study, we reported that a novel lncRNA ENST00113 (lnc00113) was significantly up-regulated through microarray analysis. Then we investigated the effect of lnc00113 on the cell apoptosis, proliferation, and migration of VSMCs and ECs, and further confirmed that the role of lnc00113 in atherosclerosis may be through regulating the PI3K/Akt/mTOR signaling pathway in vitro.

2. Materials and methods

2.1. Patients

After obtaining informed consent, we collected serum samples from 32 patients with atherosclerosis and 24 healthy volunteers from Department of Cardiology, Huaihe Hospital of Henan University from May 2016 to December 2016. This study population is clinical atherosclerosis, using the conventional definition of a coronary diameter ≥ 1.5 times the diameter of the original caliber of the artery or the adjacent segment diameter, and which is not localized (>20 mm long and/or includes more than one third of the arterial length). We excluded patients with diabetes. All research protocol was approved by the Human Ethics Committees of Henan University (IEC-T01(2.0)-2016-RES-96).

2.2. Cell culture

Human umbilical vein endothelial cells (HUVECs) and VSMCs were purchased from the Institute of Biochemistry Cell Biology (Shanghai, China). The cells were cultured in DMEM (Hyclone, South Logan, UT) with 1% penicillin/streptomycin (100 U/mL/100 mg/mL) (Beyotime, Beijing, China) and 10% fetal bovine serum (FBS, Gibco, Grand Island, NY) in a humidified incubator with 5% CO_2 and 95% air at 37°C.

2.3. Microarray analysis

Total RNA was extracted from 2 pairs of serum samples of both atherosclerosis patients and healthy volunteers. Total RNA was extracted and RNA quality was determined. Quick Amp Labeling Kit, One-Color (Agilent p/n 5190-0442) was used to carry out label reaction, then the labeled/amplified RNA was purified and labeled cRNA quality was determined. Hybridization was performed using Agilent Gene Expression Hybridization Kit (Agilent p/n 5188-5242) according to the manufacturer's protocols and the microarray was washed. Agilent microarray scanner (Agilent p/n G2565BA) was used and Agilent Feature Extraction software (version 11.0.1.1) was used to extract data. The microarray was performed by KangChen Biotech (Shanghai, China). By comparing the normalized expression levels in atherosclerosis and nonatherosclerosis serum samples, differentially expressed lncRNAs with statistical significance (T/N or N/T fold change > 1.5, $P < .05$) were identified with a paired t test.

2.4. Small interfering RNA (siRNA) transfection

Lnc00113 siRNAs (si-00113-1, si-00113-1) and a negative control (si-NC) were purchased from GenePharma (Shanghai, China). si-00113-1, si-00113-2, and si-NC were transfected into VSMCs and HUVECs cultured in 6-well plates using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. The control groups consist of 2 parts, 1 is the cells transfected with siRNA si-NC, and the other is cells without

Table 1

Primer sequences of RT-qPCR.

Gene	Forward primer sequences (5′-3′)	Reverse primer sequences (5′-3′)
Lnc-00113	AGTCGCACATCCGATTCGAC	ATCAGGCTCACGTCATCGAG
PI3K	GAAGTTGCTCTACCCAGTGTCC	GATAGCCGTTCTTTCATTTGG
Akt	ACTCATTCCAGACCCACGAC	AGCCCGAAGTCCGTTATCTT
mTOR	ACTGCTTTGAGGTCGCTATGA	TTGCCTTTGGTATTTGTGTCC
bcl-2	TGCGCTCAGCCCTGTG	GGTAGCGACGAGAGAAGTCATC
GAPDH	AGAGGCAGGGATGTTCTG	GACTCATGACCACAGTCCATGC

bcl-2 = B-cell lymphoma-2, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, mTOR = mechanistic target of rapamycin, RT-qPCR = real-time polymerase chain reaction.

any siRNA-transfection. The knock-down effect of lnc00113 in those cells was confirmed by real-time polymerase chain reaction (PCR) before further analysis.

2.5. Total RNA extraction and quantitative real-time PCR

Total RNA was extracted using the RNAiso Plus (TAKARA, Otsu, Shiga, Japan) and Trizol LS Reagent (TAKARA, Otsu, Shiga, Japan) separately according to the manufacturer's instructions. Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) was performed using PrimeScript RT reagent Kit (TAKARA, Otsu, Shiga, Japan) following the manufacturer's protocol. Each sample was down in triplicate. The mean cycle threshold (Ct) value of each target gene was normalized against Ct value of glyceraldehyde-3-phosphate dehydrogenase. The following formula: $2^{-(\text{normalized average Cts})} \times 10^4$ was used to calculate using the relative expression of mRNAs. PrimerPremier5.0 software (Premier, Canada) was used to design the qRT-PCR primers and the sequences were shown in Table 1.

2.6. Protein extraction and western blot analysis

The VSMCs and HUVECs were grown in the 6-well plate. Total proteins were extracted by radioimmunoprecipitation assay buffer lysis buffer containing phenylmethanesulfonylfluoride or phenylmethylsulfonyl fluorid (Beyotime, Beijing, China). Bicinchoninic acid assay (BCA) assay was used to determine supernatant protein levels. Then, sodium dodecyl sulfate (SDS) loading buffer was added for 5 minutes denaturation at 95°C. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted before transferring membrane, followed by the addition of 5% skim milk for closure overnight at 4°C. After membrane washing with Tris-HCl buffer solution-Tween (TBST), the primary antibodies including PI3K, Akt, mTOR, phosphorylated-PI3K (p-PI3K), phosphorylated-Akt (p-Akt) phosphorylated-mTOR (p-mTOR), bcl-2, and internal reference β-actin (all were purchased from CST Company, USA) were added respectively and incubated overnight at 4°C. Then membranes were washed with TBST and the marked secondary antibody horseradish peroxidase (HRP) was added to incubate for 1 hour at room temperature. The protein bands were revealed by electrochemiluminescence (ECL) method and imaged using a BioSpectrum Gel Imaging System (Bio-Rad, Hercules, CA).

2.7. Cell proliferation assay

Cell proliferation was detected by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. VSMCs and HUVECs transfected with si-00113 and si-NC were seeded in 96-well culture plates and 20 μL MTT solution (5 g/L) was added to each tested well from 1st day to 5th day after cultivation. Then cultivation was continued for another 4 hours at 37°C. After that, culture solution was discarded and 20 μL DMSO was added to each tested well. A microtiter plate reader (Thermo Electron Corporation, Vantaa, Finland) was used to detect the optical density (OD) of each tested well.

2.8. Caspase 3 enzyme-linked immunosorbent assay assay

Cell apoptosis was detected by Caspase 3 ELISA assay. VSMCs and HUVECs transfected with si-00113 and si-NC were seeded in 96-well culture. Forty-eight hours later, Caspase 3 Colorimetric Protease Assay Kit (Invitrogen, Carlsbad, CA) was used to detect caspase 3 activity of target cells according to the manufacturer's instructions. OD values were measured using a microplate reader (Bio-Rad).

2.9. Wound healing assay

Cell migration was detected by wound healing assay. VSMCs and HUVECs transfected with si-00113 and si-NC were seeded in 6-well plate and cultured in incubator to get 100% gather before transfection. At the 0 hour time point, a wound was created with a pipette tip. Then the cells were washed twice and cultured in medium without FBS. Nikon Eclipse TS100 Microscope (Nikon, Japan) was used to take pictures at the 0 and 48 hours postinjury time points.

2.10. Statistical analysis

All assays were performed in triplicate and all data were expressed as the means ± standard deviation (SD). Student *t* test was employed to determine the significance of 2 group differences. One-way analysis of variance analysis was used for multiple comparisons. All statistical tests were done using SPSS 19.0 (SPSS Inc, Chicago, IL). The $P < .05$ was considered to be significant.

3. Results

3.1. Differently expressed lncRNAs by microarray analysis

We detected 430 differentially expressed lncRNA transcripts between atherosclerosis and normal serum samples with 243 up-regulated lncRNAs and 187 down-regulated lncRNAs (Fig. 1, Supplementary Figure 1, http://links.lww.com/MD/C201). Here, we defined those with normalized expression levels consisting of fold changes greater than 1.5 (i.e., T/N > 1.5 or N/T > 1.5) as significantly differentially expressed lncRNAs. As shown in Table 2, the 20 most significantly differentially expressed lncRNA transcripts are listed, with lnc00113 being the top significantly up-regulated. Therefore, lnc00113 was chosen to further explore its roles in atherosclerosis through following experiments.

3.2. Lnc00113 was highly expressed in atherosclerosis patients

To further verify the role of lnc00113 in atherosclerosis, we first measured the expression of lnc00113 in the serum of patients with atherosclerosis. The results showed that the expression of

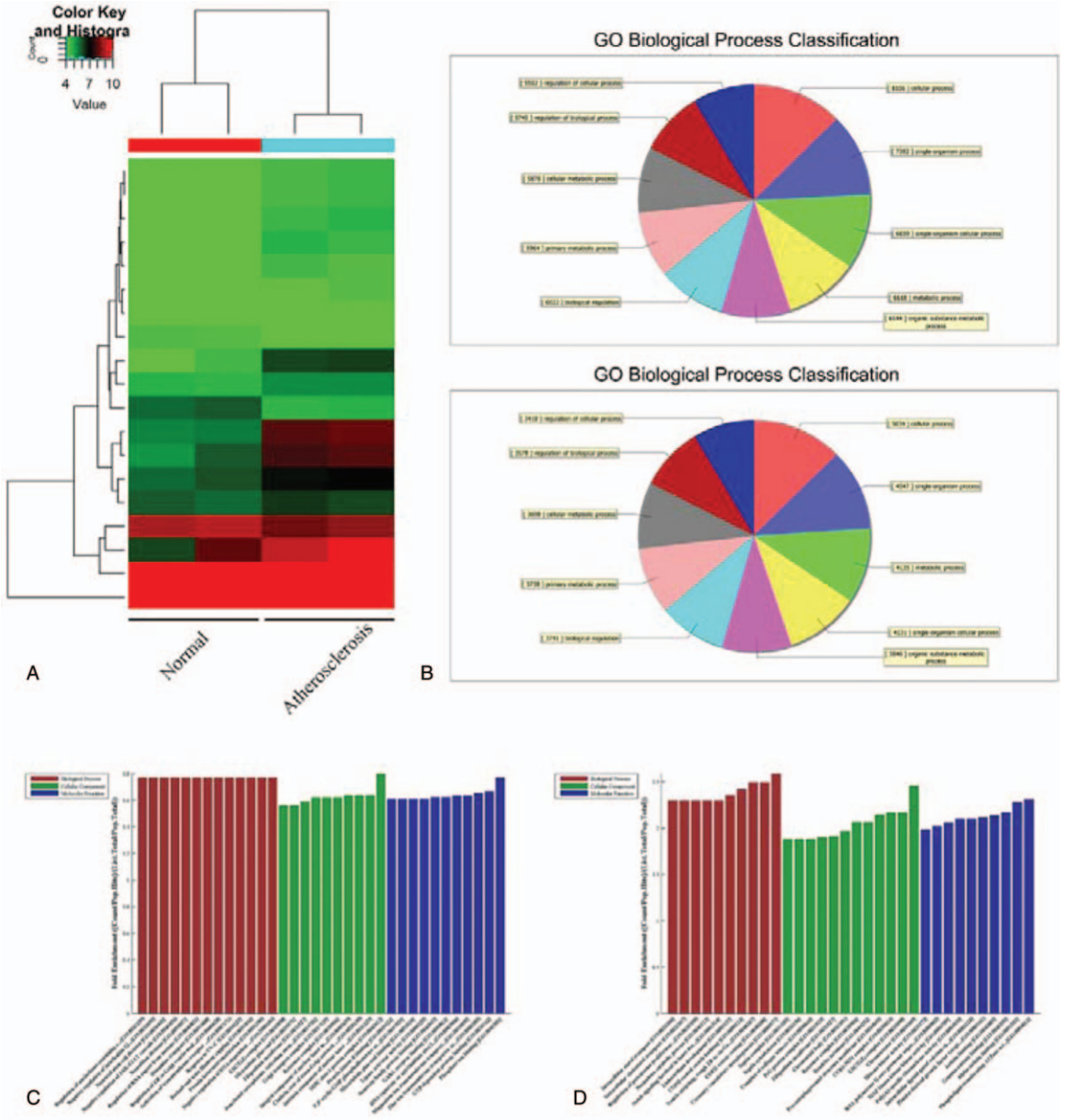

Figure 1. Significantly aberrantly expressed lncRNAs. A, Heat map of distinguishable mRNA expression amid atherosclerosis and normal serum samples. Red shows relatively high expression while green shows relatively low expression. B, GO biological process classification for aberrantly expressed mRNAs. The upper graph was related to the up-regulated mRNAs while the lower one was related to the down-regulated mRNAs. C, GO analysis indicates the top enrichment score in the biological process, cellular components, molecular function of up-regulated mRNAs. D, GO analysis indicates the top enrichment score in the biological process, cellular components, molecular function of down-regulated mRNAs.

Table 2

Significant differentially expressed lncRNAs by microarray analysis.

Up-regulated			Down-regulated		
lncRNAs	**Log_2 fold change (T/N)**	***P* values**	**lncRNAs**	**Log_2 fold change (T/N)**	***P* values**
ENST00113	−5.621	.011	ENST0003318	3.674	.028
BC07016	−4.547	.026	NR_040167	3.106	.016
NR_062805	−4.282	.039	DQ167004	2.937	.038
ENST00987533	−3.691	.023	AL003176	2.615	.005
uc001wik.3	−3.449	.036	ENST00110763	2.497	.029
NR_098365	−3.235	.006	ENST00476791	2.305	.036
CR887531	−3.056	.035	uc006ajw.2	2.249	.021
uc002ckj.2	−2.836	.037	uc001ckz.1	2.168	.017
ENST00386212	−2.794	.026	NR_042820	2.103	.032
AK023048	−2.648	.017	BC013286	2.007	.008

lncRNAs = long noncoding RNAs, T/N = tumor samples (T) relative to corresponding healthy serum samples (N).

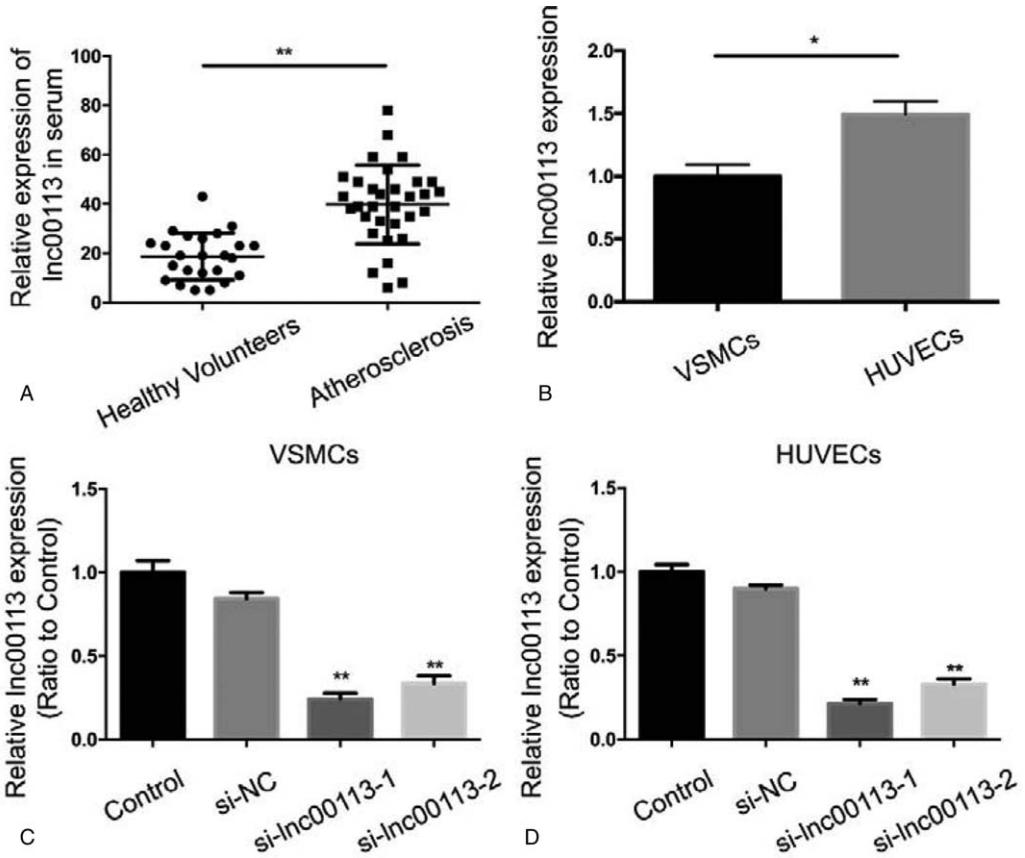

Figure 2. The expression of lnc00113. A, The expression of lnc00113 was significantly up-regulated in the atherosclerosis serum samples compared with healthy serum samples ($^{**}P < .01$). B, The expression of lnc00113 in vascular smooth muscle cells (VSMCs) and human umbilical vein endothelial cells (HUVECs) ($^{*}P = .03$). C, The expression of lnc00113 in VSMCs transfected with si-00113-1 and si-00113-2 was remarkedly decreased compared with si-NC transfected cells and control cells ($^{**}P < .01$). D, The expression of lnc00113 in HUVECs transfected with si-00113-1 and si-00113-2 was remarkedly decreased compared with si-NC transfected cells and control cells. $^{**}P < .01$.

lnc00113 was significantly up-regulated in the serum when compared with healthy serum samples. (Fig. 2A). These observations indicate that lnc00113 may probably be a risk factor for atherosclerosis.

3.3. Down-regulated expression of lnc00113 after siRNA transfection

To detect the expression of lnc00113 in VSMCs and HUVECs after transfection of siRNA for 24 to 48 hours, qRT-PCR was employed. We first detect the expression of lnc00113 in both VSMCs and HUVECs and they are differently expressed (Fig. 2B). Further, we found that the expression of lnc00113 in VSMCs and HUVECs transfected with si-00113-1 and si-00113-2 was remarkedly decreased compared with si-NC transfected cells and control cells (Fig. 2C and D).

3.4. Lnc00113 promotes proliferation of VSMCs and HUVECs

MTT assay was used to detect whether lnc00113 influenced the proliferation ability of VSMCs and HUVECs in vitro. The results showed that proliferation ability was markedly suppressed after transfection with si-00113-1 and si-00113-2 compared with si-NC-transfected cells and control cells (Fig. 3A and B). These data reveal that that lnc00113 could promote proliferation of VSMCs and HUVECs, which may possibly speed up the development of atherosclerosis.

3.5. Lnc00113 inhibits apoptosis of VSMCs and HUVECs

Caspase 3 ELISA assay was used to detect the apoptosis ability of VSMCs and HUVECs after transfection treatment in vitro. The results showed that the apoptosis ability was significantly promoted in both cell lines after transfection with si-00113-1 and si-00113-2 compared with si-NC-transfected cells and control cells (Fig. 3C and D). These results indicate that lnc00113 could suppress apoptosis of VSMCs and HUVECs, implying that lnc00113 may be an important factor in the process of atherosclerosis.

3.6. Lnc00113 promotes migration of VSMCs and HUVECs

Wound healing assay was used to measure whether lnc00113 had an effect on the migration ability of VSMCs and HUVECs in vitro. We found that lnc00113 down-regulation in VSMCs and HUVECs cells transfected with si-00113-1 or si-00113-2 significantly suppressed cell migration ability compared with si-NC transfected cells and control cells (Fig. 4A and B). These observations demonstrate that lnc00113 promotes VSMCs and HUVECs migration, which is likely to accelerate the formation of atherosclerosis.

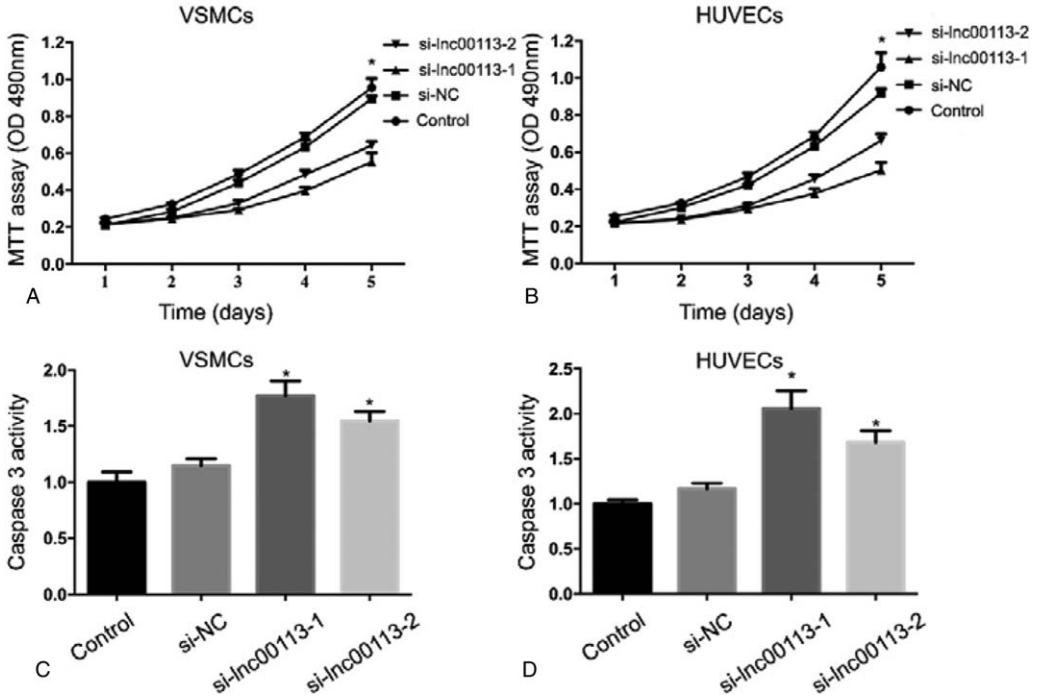

Figure 3. lnc00113 promotes proliferation and survival of VSMCs and HUVECs. A and B, Proliferation ability of VSMCs and HUVECs was significantly suppressed after transfection with si-00113-1 and si-00113-2 compared with si-NC-transfected cells and control cells. ($^{*}P = .03$ for (A) and $^{*}P = .03$ for (B)). C and D, The apoptosis ability of VSMCs was significantly promoted after transfection with si-00113-1 and si-00113-2 compared with si-NC-transfected cells and control cells. ($^{*}P = .02$ and $^{*}P = .03$ respectively). D, The apoptosis ability of HUVECs was significantly promoted after transfection with si-00113-1 and si-00113-2 compared with si-NC-transfected cells and control cells. ($^{*}P = .02$ and $^{*}P = .03$ respectively). HUVECs = human umbilical vein endothelial cellslinc, VSMCs = vascular smooth muscle cells.

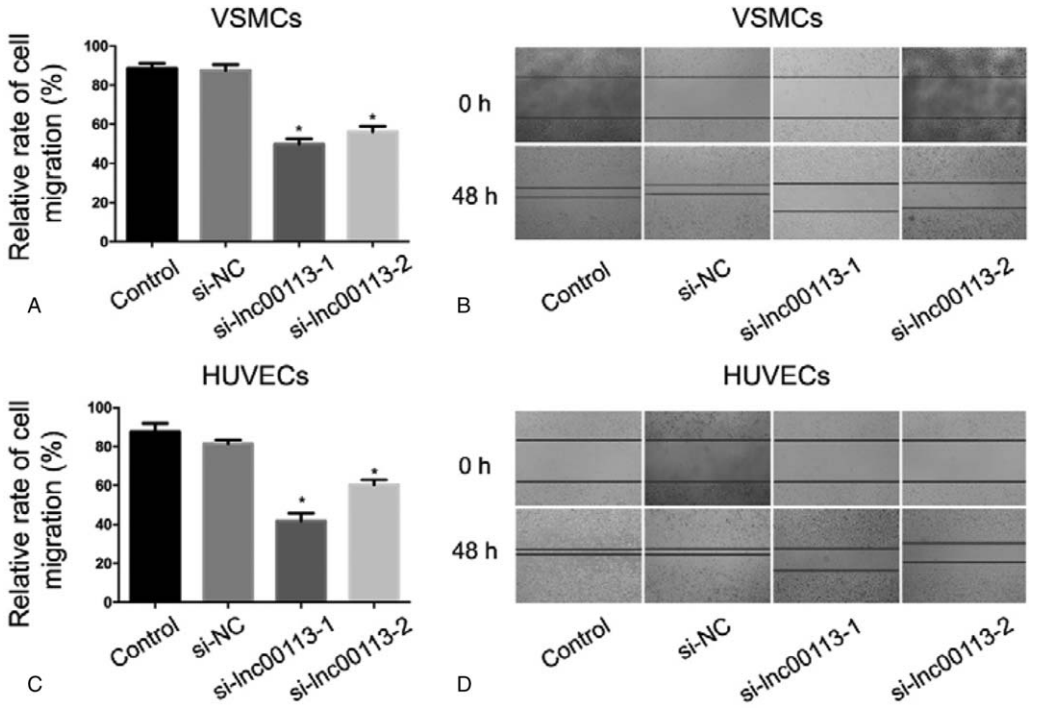

Figure 4. lnc00113 promotes migration of VSMCs and HUVECs. A to D, Migration ability of VSMCs ($^{*}P = .03$ and $^{*}P = .02$ respectively) and HUVECs ($^{*}P = .03$ and $^{*}P = .02$ respectively) was remarkably down-regulation after transfection with si-00113-1 or si-00113-2 compared with si-NC transfected cells and control cells. HUVECs = human umbilical vein endothelial cellslinc, VSMCs = vascular smooth muscle cells.

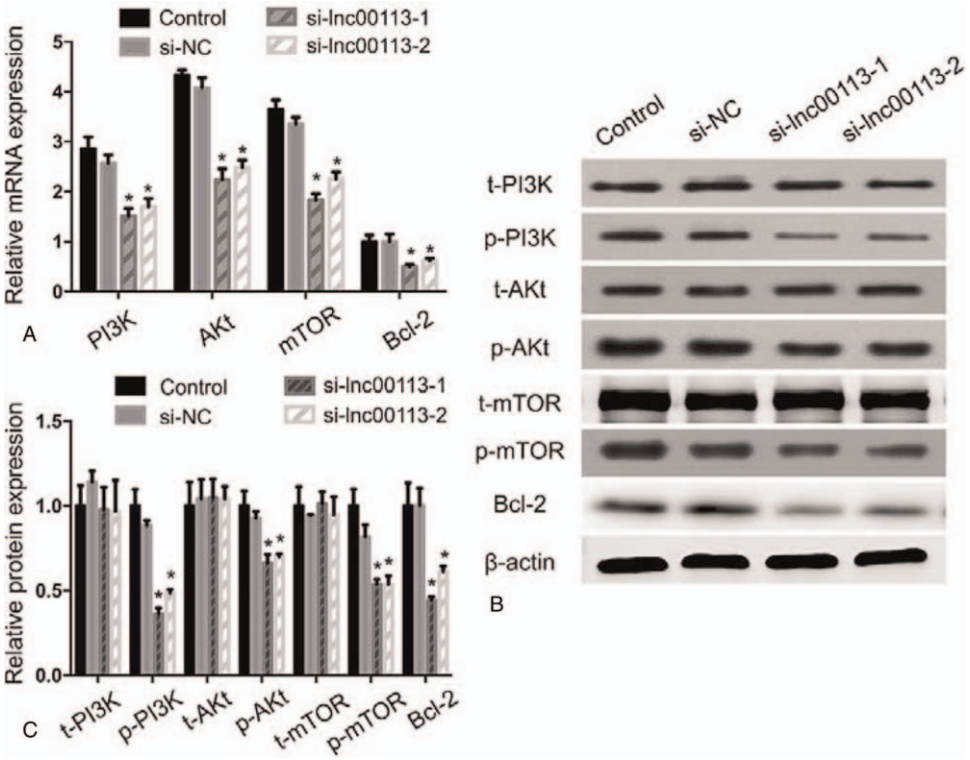

Figure 5. lnc00113 activates PI3K/Akt/mTOR signaling pathway of HUVECs. A, The mRNA expressions of PI3K ($^{*}P=.03$ and $^{*}P=.03$ respectively), Akt ($^{*}P=.03$ and $^{*}P=.03$ respectively), mTOR ($^{*}P=.02$ and $^{*}P=.03$ respectively) and bcl-2 ($^{*}P=.03$ and $^{*}P=.03$ respectively) were significantly decreased in HUVECs cells transfected with si-00113-1 or si-00113-2 compared with si-NC-transfected cells and control cells. B and C, The protein expressions of phosphorylated-PI3K (p-PI3K) ($^{*}P=.02$ and $^{*}P=.02$ respectively), phosphorylated-Akt (p-Akt) ($^{*}P=.03$ and $^{*}P=.03$ respectively), phosphorylated-mTOR (p-mTOR) ($^{*}P=.03$ and $^{*}P=.03$ respectively), and bcl-2 ($^{*}P=.03$ and $^{*}P=.03$ respectively) in HUVECs cells transfected with si-00113-1 or si-00113-2 were dramatically decreased compared with si-NC-transfected cells and control cells, while the total-PI3K (t-PI3K), total-Akt (t-Akt), and total-mTOR (t-mTOR) protein expressions were not changed. HUVECs = human umbilical vein endothelial cellslinc.

3.7. Lnc00113 activates PI3K/Akt/mTOR signaling pathway of VSMCs and HUVECs

qRT-PCR and western blot assays were used to detect the effect of lnc00113 on PI3K/Akt/mTOR signaling pathway. As the results show, the mRNA expressions of PI3K, Akt, mTOR, and bcl-2 were significantly decreased in HUVECs cells transfected with si-00113-1 or si-00113-2 compared with si-NC-transfected cells and control cells (Fig. 5A). The protein expressions of p-PI3K, p-Akt, p-mTOR, and bcl-2 in HUVECs cells transfected with si-00113-1 or si-00113-2 were dramatically decreased compared with si-NC-transfected cells and control cells. However, the total-PI3K (t-PI3K), total-Akt (t-Akt), and total-mTOR (t-mTOR) protein expressions were not changed (Fig. 5B and C). These results indicate that lnc00113 is positively correlated with PI3K/Akt/mTOR signaling pathway in HUVECs. PI3K/Akt/mTOR signaling pathway is activated in the development of atherosclerosis. However, whether lnc00113 promotes proliferation, survival, and migration in atherosclerosis directly by activating PI3K/Akt/mTOR signaling pathway remains to be elucidated further.

4. Discussion

As one of the most common vascular disorders, atherosclerosis is the root cause of a series of diseases including stroke, myocardial infarction, and gangrene.[4] EC and VSMC are 2 critical factors in cardiovascular system, whose dysfunction may lead to the formation of atherosclerosis. Under normal physiological conditions, the ECs are capable of adapting to the environment like hyperlipidemia and inflammation stimulus by generating new ECs to replace the damaged one. However, in atherosclerosis, persistent injury could induce EC injury and apoptosis.[19,20] VSMCs accumulation is another major reason leading to atherosclerosis. Activated VSMCs may possibly contribute to the progression and eventual rupture of atherosclerotic plaques.[5,21] Thus, there is an urgent need to find effective therapeutic target to reduce the incidence of atherosclerosis. In the present study, we found that lnc00113 is highly expressed in the serum of patients with atherosclerosis. It could promote proliferation and migration, suppress apoptosis by activating PI3K/Akt/mTOR signaling pathway in atherosclerosis, which may provide therapeutic methods for atherosclerosis. Gene chips and other high-throughput sequencing tools have received increasing attention, especially in cancer research. Huang[15] found that lncRNA HOXC-AS1 and HOXC6 were down-regulated in carotid atherosclerosis by microarray analysis. Singh[22] assessed the expression profiles of lncRNAs and protein-coding mRNAs in endothelial cells 24 hours after stimulation of oxidized low-density lipoprotein (100 μg/mL) with Arraystar human lncRNA expression microarray V3.0. A total of 30,584 lncRNAs were screened out, 923 significantly up-regulated, and 975 significantly down-regulated in response to oxLDL exposure. At the same time the number of lncRNAs associated with atherosclerosis remains unknown. To explore the

mode of expression of lncRNAs in atherosclerosis, we used microarrays to assess the lncRNA profile between serum samples from atherosclerosis and healthy volunteers. Hereafter, we chose the lnc00113 that was up-regulated to further validate its role in atherosclerosis.

The role of microRNAs has been extensively studied in atherosclerosis. NovákJ has reviewed synthetically the intracellular and extracellular functions of the most important microRNAs involved in atherosclerotic pathophysiology such as miR-27a/b, miR-33/33* and so on. At present, more and more literature shows that lncRNAs participates in gene transcription and transcription.[23] Recent studies have demonstrated that lncRNAs are involved in the regulation of atherosclerosis. A new study shows that lncRNA-H19 was highly expressed in atherosclerosis by regulating MAPK/NF-kB signaling pathway, which could be used as a potential target for atherosclerosis.[24] Another experiment reported that lncRNA-RNCR3 was highly expressed in mouse and human aortic atherosclerosis. The proliferation and migration in ECs and VSMCs was suppressed when lncRNA-RNCR3 decreased, implying that lncRNA-RNCR3 could be a potential atherosclerosis target.[25] Wu[26] discovered a novel regulatory factor, lincRNA-p21, which regulates neointima formation, proliferation, apoptosis, and atherosclerosis in VSMCs by enhancing p53 activity, suggesting that lncRNA may serve as a therapeutic for atherosclerosis and related heart therapeutic targets for vascular disease. In this study, it is our first time to identify an interaction between lnc00113-associated atherosclerosis in vitro. In the serum of atherosclerosis, the expression of lnc00113 was significantly increased compared with control group. Furthermore, the down-regulation of lnc00113 can significantly suppress proliferation, migration, and survival of VSMCs and HUVECs, indicating that lnc00113 may play a promoter role in the formation of atherosclerosis.

PI3K/Akt/mTOR signaling pathway has a variety of cell functions, including proliferation, differentiation, survival, and invasion, lncRNAs are closely related. A report has shown that selective inhibition of the Akt/mTOR pathway inhibits the progression of atherosclerosis and enhances the stability of atherosclerotic plaques by activating macrophage autophagy.[27] Investigator pointed out that mTOR pathway is activated by atherosclerotic oxLDL through PI3K/Akt and is required for the proliferation of smooth muscle cells. In addition, resveratrol specifically blocks this pathway, thereby inhibiting oxLDL-induced SMC proliferation, highlighting the new properties of resveratrol can contribute to the general anti-atherosclerotic properties of the polyphenols.[28] Lee[29] discovered naringin treatment can block TNF-α-induced VSMC PI3K/AKT/p70S6K pathway, which provides a theoretical basis for the prevention of atherosclerosis. In view of the promotion of PI3K/Akt/mTOR pathway in atherosclerosis, lnc00113 can activate PI3K/Akt/mTOR pathway, suggesting that inactivation of lnc00113 or PI3K/Akt/mTOR pathway may suppress clinical development and progression of atherosclerosis.

5. Conclusion

We identified an important role of lnc00113 in VSMCs and HUVECs that promotes cell proliferation and migration, suppresses apoptosis by activating PI3K/Akt/mTOR signaling pathway, which could probably serve as a promising therapeutic target for atherosclerosis.

Author contributions

Conceptualization: Xinliang Yao.
Data curation: Chengyun Yan.
Formal analysis: Chengyun Yan.
Funding acquisition: Qinlin Wan.
Investigation: Xinliang Yao, Lei Zhang, Yanming Li.
Project administration: Xinliang Yao.
Resources: Xinliang Yao.
Software: Chengyun Yan.
Supervision: Qinlin Wan.
Writing – original draft: Xinliang Yao.

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