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ORIGINAL ARTICLE

Linc00299/miR-490-3p/AURKA axis regulates cell growth and migration in atherosclerosis

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Abstract

Long non-coding RNA (lncRNA) plays a crucial role in regulating various cellular processes in atherosclerosis. The present study identified the regulation of Linc00299, via miR-490-3p targeting Aurora kinase A (AURKA), on migration and proliferation of endothelial cells and vascular smooth muscle cells (VSMCs) during atherosclerosis. The expression of RNAs was assessed by real-time PCR. The proliferation, apoptosis and migration were detected using MTT assay, Annexin V/PI staining and Transwell system, respectively. Bindings of Linc00299/miR-490-3p and subsequent miR-490-3p/AURKA were verified by luciferase and biotin pull-down assays. The protein expression of AURKA was detected by Western blotting. Expressions of Linc00299 and miR-490-3p were upregulated and downregulated in atherosclerosis patients, respectively. Both Linc00299 knockdown and miR-490-3p overexpression suppressed cell proliferation, increased apoptosis and inhibited migration of VSMCs and HUVECs. Linc00299 directly bound to miR-490-3p which targeted AURKA. The regulation of Linc00299 on expression of AURKA and proliferation and migration of VSMCs were dependent on miR-490-3p. Atherosclerosis-increased Linc00299 acts as a sponge of miR-490-3p to upregulate AURKA, and as a result increases proliferation and migration in VSMCs and HUVECs. Our study reveals an important effect of Linc00299/miR-490-3p/AURKA axis on regulating cell proliferation and migration in atherosclerosis.

Keywords Atherosclerosis · Linc00299 · miR-490-3p · AURKA · Vascular smooth muscle cells

Introduction

Atherosclerosis is the main cause of cardiovascular diseases [1]. Although significant progress has been achieved in understanding atherosclerosis progress, identifying risk factors and implementing therapies, atherosclerosis remains as the major cause of death worldwide. Hence, there is still an urgent need to provide novel therapeutic targets.

It is well recognized that atherosclerosis is a chronic inflammatory and metabolic disorder involving multiple cellular biological processes [2, 3]. Endothelial cells, macrophages and vascular smooth muscle cells (VSMCs) are crucial players in the pathological progress of this disease, especially the pathogenesis of atherosclerotic plaques. During atherosclerosis, hyperlipidemia and chronic vascular inflammation can induce apoptosis, inhibit proliferation and promote dysfunction in endothelial cells [4]. Endothelial dysfunction increases the levels of vascular cell adhesion molecules and chemokines which recruit monocytes, T cells and platelets [5]. Upon stimulation of proinflammatory cytokines and chemokines, monocytes at the lesion site differentiate into macrophages that enhance the local inflammation and then trigger excessive proliferation and migration of VSMCs to cover the atherosclerotic core [3]. In the late stage of atherosclerosis, apoptosis in the VSMCs causes plaque instability and thrombosis, which lead to blockage in the arteries and eventually result in cardiovascular diseases and ischemic stroke. Therefore, regulation and stabilization of endothelial cells and VSMCs suggest a promising therapeutic strategy for atherosclerosis [6, 7].

Long noncoding RNAs (lncRNAs) are RNAs of more than 200 nucleotides in length and do not code proteins [8]. LncRNAs have been identified to be important regulators of gene transcriptions in various diseases including cancers and vascular diseases [9]. LncRNAs can directly bind to miRNAs and serve as a decoy or sponge to negatively regulate the expression of miRNAs [10]. More and more lncRNAs

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have been identified and characterized as an important regulator interfering with the progress of atherosclerosis, including endothelial inflammatory activation, apoptosis and proliferation of endothelial cells and VSMCs and inflammatory activation of macrophages [11, 12]. Hence, lncRNAs may be promising therapeutic targets for treatment of atherosclerosis.

LncRNA Linc00299 is a noncoding gene on chromosome 2 and is proposed to be a causative factor for neurodevelopmental disabilities [13]. The role of Linc00299 in pathological progress of atherosclerosis remains to be revealed. This study investigated the function of Linc00299 in regulating proliferation, apoptosis and migration of VSMCs and endothelial cells during atherosclerosis and the potential mechanisms.

Materials and methods

Clinical samples

The study was approved by the Ethics Committee of Affiliated Hospital of Hebei University. All clinical subjects in the study were patients with atherosclerosis and healthy volunteers between September 2016 and May 2017 at the Affiliated Hospital of Hebei University. Written informed consent had been signed by all patients. Serum samples were collected from atherosclerosis patients and healthy volunteers. Atherosclerosis plaques and healthy arteries were obtained from patients who underwent carotid endarterectomy.

Cell cultures

Primary human VSMCs were purchased from Lonza Corporation (Allendale, NJ, USA) and maintained in DMEM supplemented with 10% fetal bovine serum (FBS). Human umbilical vein endothelial cells (HUVECs, Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM with 10% FBS and 1% endothelial cell growth supplement. Cells were placed in a 37 °C humidified incubator with 5% CO_2 and used from passage 4–6.

RNA extraction and real-time PCR

Total RNA of human and cell samples was obtained using TRIzol (TaKaRa, Dalian, China) and cDNA was reverse-transcribed using Prime Script RT Master Mix kit (TaKaRa). Real-time PCR was performed on ABI 7500 system (Thermo Fisher Scientific, Carlsbad, CA, USA) with SYBR Green PCR kit (TaKaRa), as per the manufacturer's instructions. The primers used are listed as follows: Linc00299 (forward) ACAGCCAGAAACAAGATAAGCG (reverse) CAGGAAGATGGTCCCAAAGAA; GAPDH (internal control for Linc00299, forward) ACAACTTTGGTATCGTGGAAGG (reverse) GCCATCACGCCACAGTTTC. MiR-490-3p (forward) AACATGCCATGGGGGCCGGAGCGGAGT (reverse) GCTGTCAACGATACGCTACGT; U6 (internal control for miR-490-3p, forward) TGCGGGTGCTCGCTTCGGCAGC (reverse) GTGCAGGGTCCGAGGT; AURKA (forward) CAGGCTCAGCGGGTCTTGT (reverse) TACCCAGAGGGCGACCAAT; GAPDH (internal control for AURKA, forward) ACAACTTTGGTATCGTGGAAGG (reverse) GCCATCACGCCACAGTTTC. 2^{-CT} was used to present the relative expression of RNA levels.

Cell transfection

Cells were cultured in 6-well plates at a density of 5×10^6/well. Knockdown of Linc00299 was achieved by transfection with shRNAs targeting Linc00299 (Linc00299 shRNA1 forward: CCGGGGGTTCGATGGTTCAAAGAGAGGATCCTCTCTTTGAACCATCGAACCCTTTTTG, reverse: AATTCAAAAAGGGTTCGATGGTTCAAAGAGAGGATCCTCTCTTTGAACCATCGAACCC; Linc00299 shRNA2 forward: CCGGGGCAAGTGGATTGGAGGTCTTGGATCCAAGACCTCCAATCCACTTGCCTTTTTG, reverse: AATTCAAAAAGGCAAGTGGATTGGAGGTCTTGGATCCAAGACCTCCAATCCACTTGCC) or empty vector (GenePharma, Shanghai, China). Overexpression of AURKA in VSMCs was achieved by transfection with pSin-vec, pSin-Linc00299 (forward: CTAGAATTCGACACTGAGCTAGGGTG, reverse: CTAGGATCC TAAATGAAAAAGAGGGAGGTT), or pSin-Linc00299 plus miR-490-3p mimics. The transfections were conducted using Lipofectamine 2000 (Invitrogen, Waltham, MA USA) as per the manufacturer's instructions. Subsequent experiments were conducted 24 h after transfection.

Cell proliferation detection

Cell proliferation was assessed using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay from day 1 to 5 after transfection. MTT (Sigma Aldrich, Sigma Aldrich, St. Louis, MO, USA) was added at a final concentration of 0.5 mg/ml/well and incubated for 4 h at 37 °C. The medium was removed. Formazan crystals were dissolved in dimethyl sulfoxide and then absorbance was measured at 570 nm.

Cell apoptosis detection

Cell apoptosis was determined using an FITC–Annexin V/PI apoptosis kit (BD Pharmingen, Franklin Lakes, NJ, USA) as per the manufacturer's protocols. Cells were harvested at a density of 5×10^5/ml, washed in PBS and then incubated

in 20 μg/ml FITC–Annexin V and 50 μg/ml PI for 15 min at room temperature in dark. The apoptosis rate was measured using FACS Calibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and analyzed using FlowJo 10 (Tree Star, Inc., Oregon, USA).

Cell migration assay

VSMCs and HUVECs migration ability was evaluated using a transwell chamber system (8 μm, Corning Costar, Cambridge, MA, USA) as previously described [14]. Briefly, the cells were cultured with DMEM in the upper chamber of transwell compartments at a density of 5×10^4/well. The lower chamber was filled with DMEM containing 10% FBS. After incubation for 12 h, cells which migrated to the underside of the filter were fixed with 4% paraformaldehyde (Sigma Aldrich, St. Louis, MO) and then stained with hematoxylin (Sigma Aldrich). Migrated cells were counted in three randomly chosen fields under a light microscope.

Luciferase reporter assay

Luciferase reporter assay was conducted as reported [15]. To predict the potential binding sites of miR-490-3p in Linc00299, bioinformatics analysis was conducted using RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid). Luciferase reporter psiCHECK™-2 plasmids (Promega, Madison, WI, USA) carrying the predicted wild type (Linc00299-WT; forward: CTACTCGAG GACACTGAG CTAGGGTG, reserve: CTAGCGGCCGC TAAATGAAA AAGAGGGAGGTT), mutated binding sites of miR-490-3p in Linc00299 (Linc00299-MUT; forward: CCTGGCTCC ACCCTTAGCGGATTTTA, reverse: TAAAATCCGCTA AGGGTGGAGCCAGG), or AURKA (forward: CTACTC GAGAAAGGACCCAGCACATCTGCTGGA, reverse: CTAGCGGCCGC TCTGTACATATATCTTTATTTTCA TAC) were constructed. Cells were cultured at a density of 3×10^4/well in 24-well plates and co-transfected with the constructed luciferase reporter vector, pRL–TK (Promega) and miR-490-3p or miR-NC using Lipofectamie 2000 (Invitrogen). Luciferase activities were measured using the Dual-Luciferase Reporter assay system 48 h after transfection (Promega).

Biotin pull-down assay

Biotin pull-down was conducted as previously reported [16, 17]. Lysates from VSMCs and HUVECs were incubated with biotin-labeled sense or antisense DNA oligomers corresponding to Linc00299. The biotin-labeled DNA oligomers were as follows: Linc00299-DNA-1-sense: (biotin-) CGGATCACCTAGAGACTGGAAATGCTTTCT, Linc00299-DNA-1-antisense: (biotin-) AGAAAGCATTTC CAGTCTCTAGGTGATCCG; Linc00299-DNA-2-sense: (biotin-)CAGAGATGCTTGGAGAAGGCTGAGGTCTCC, Linc00299-DNA-2-antisense: (biotin-) GGAGACCTCAGC CTTCTCCAAGCATCTCTG; Linc00299-DNA-3-sense: (biotin-)CCACCCCTCTCAAGTTTTATTTTCCGACGG; Linc00299-DNA-3-antisense: (biotin-) CCGTCGGAAAAT AAAACTTGAGAGGGGTGG. The reaction mix was incubated with streptavidin-coupled agarose beads (Invitrogen) and incubated at 4 °C for 4 h. The beads were washed and resuspended in TRIzol to extract RNAs for real-time PCR.

Western blotting

Total protein was isolated from cells after transfection for 48 h. Protein concentration was determined using the BCA method (Thermo Fisher Scientific). Proteins were separated by electrophoresis in 10% SDS PAGE gels and transferred onto a PVDF membrane (Millipore, Billerica, MA, USA). After blocking using 5% skimmed milk, the PVDF membranes were incubated overnight with primary antibodies specific for AURKA and -actin (Abcam, Cambridge, UK) at 4 °C, followed by incubation with appropriate HRP-conjugated secondary antibodies. Chemiluminescence was developed and detected by ChemiDoc™ Touch Imaging System (Bio-Rad, Hercules, CA, USA).

Statistical analysis

Statistical analyzes were performed using GraphPad Prism 7. Data are expressed as mean ± SD. To evaluate significant differences between two groups, Student's *t* test was used. Two-way ANOVA was performed to analyze the proliferation rate. Differences were statistically significant at $P<0.05$.

Results

Linc00299 and miR-490-3p were dysregulated in atherosclerosis patients

The transcription levels of Linc00299 and miR-490-3p were evaluated by real-time PCR in the serum of atherosclerosis patients versus healthy controls and in atherosclerotic plaques versus healthy human arteries. The transcription levels of Linc00299 and miR-490-3p were significantly higher and lower, respectively, in the serum from atherosclerosis patients compared with those in the serum from healthy volunteers (Fig. 1a, b). Consistent with the changes in the serum, the transcription levels of Linc00299 and miR-490-3p were significantly upregulated and downregulated, respectively, in the atherosclerotic plaques compared with those in healthy human arteries (Fig. 1c, d).

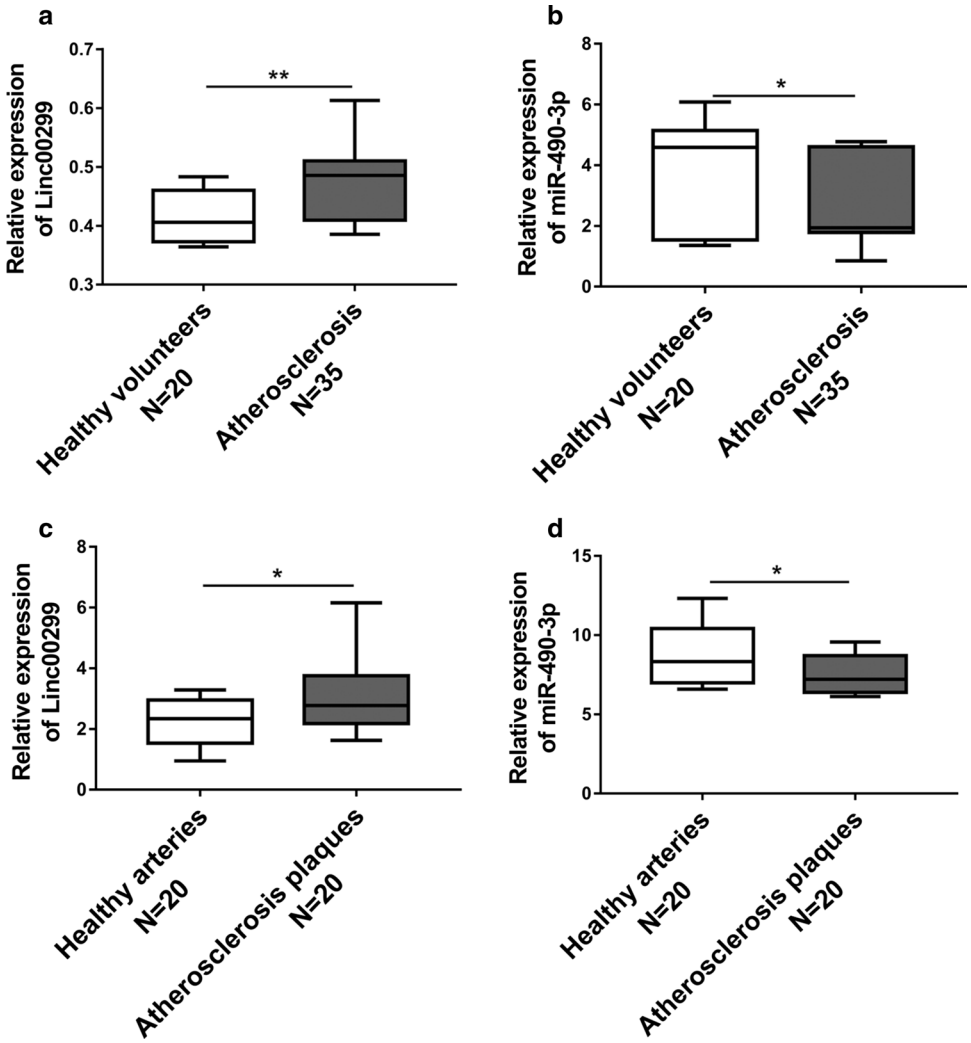


Fig. 1 Linc00299 and miR-490-3p were dysregulated in atherosclerosis patients. **a**, **b** The expression of Linc00299 was significantly up-regulated but miR-490-3p was significantly down-regulated in the atherosclerosis serum samples compared with healthy serum samples. **c**, **d** The expression of Linc00299 and miR-490-3p were measured in healthy human arteries and atherosclerotic plaques by realtime PCR. The data represent the mean ± SD from three independent experiments. *$P < 0.05$; **$P < 0.01$

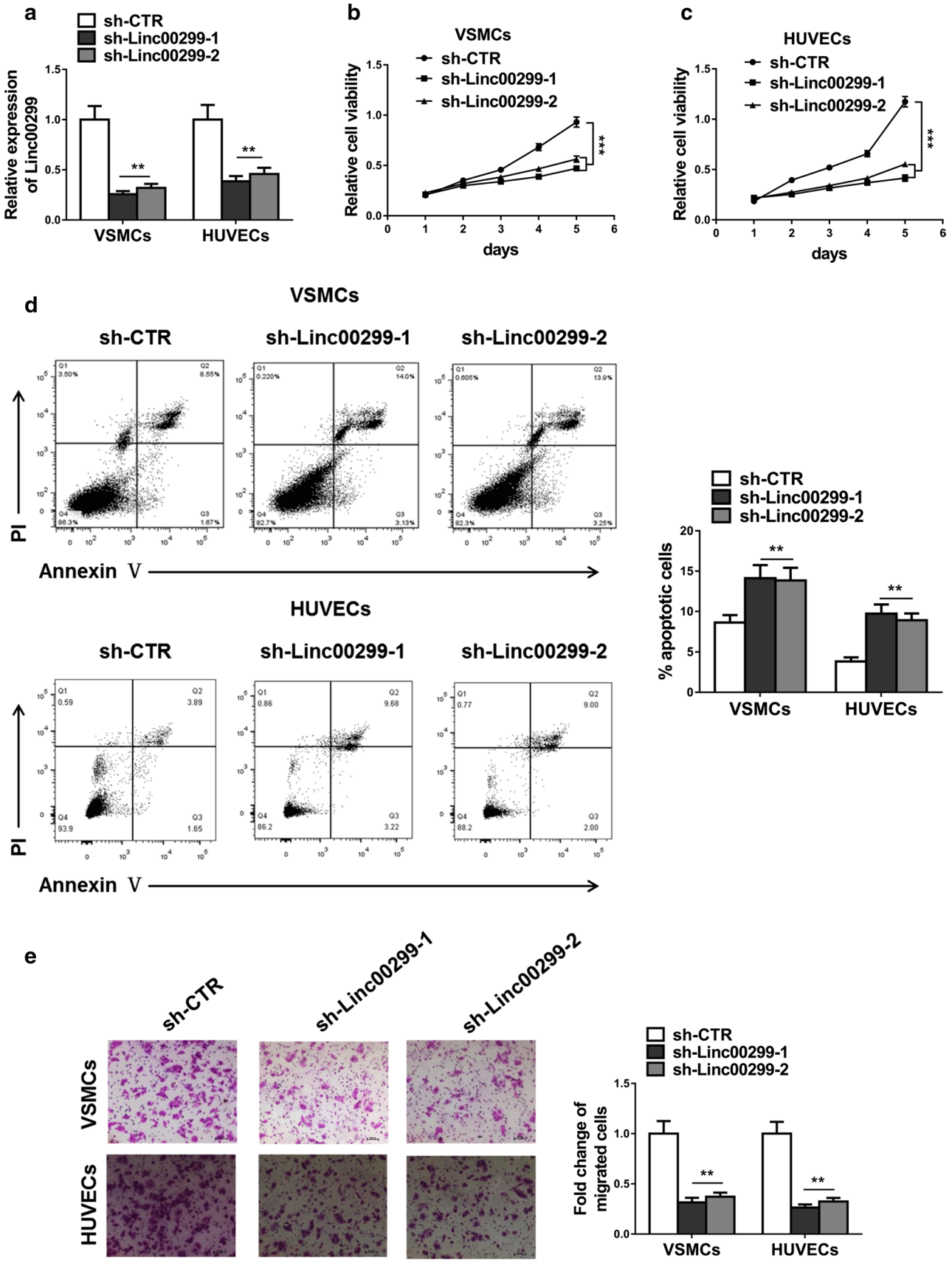
Knockdown of Linc00299 inhibited proliferation and migration and promoted apoptosis in VSMCs and HUVECs

VSMCs and HUVECs were stably transfected with Linc00299 shRNAs. As confirmed by real-time PCR, the transcription level of Linc00299 was robustly knocked down by either sh-Linc00299-1 or sh-Linc00299-2 compared with empty vector (sh-CTR) in both cells (Fig. 2a). Knockdown of Linc00299 markedly suppressed the proliferation rate of VSMCs and HUVECs for at least 5 days after transfection (Fig. 2b, c). In addition, Linc00299 knockdown significantly increased the apoptosis in both cells as assessed by Annexin V/PI staining (Fig. 2d). The migration ability of VSMCs and HUVECs was evaluated by the transwell system. As shown in Fig. 2e, knockdown of Linc00299 remarkably inhibited the migration ability of both cells.

Linc00299 negatively regulated and directly bound to miR-490-3p

Linc00299 knockdown significantly increased the expression of miR-490-3p in both VSMCs and HUVECs

Fig. 2 Downregulation of Linc00299 inhibited proliferation and migration of VSMCs and HUVECs. **a** Transcription level of Linc00299 was determined by realtime PCR in VSMCs and HUVECs stably transfected with Linc00299 shRNAs (sh-Linc00299-1 and sh-Linc00299-2) or empty vector (sh-CTR). **b**, **c** Proliferation ability of VSMCs and HUVECs was significantly suppressed after transfection with Linc00299 shRNAs (sh-Linc00299-1 and sh-Linc00299-2) compared with empty vector (sh-CTR). **d** Knockdown of Linc00299 significantly promoted apoptosis. Apoptosis of VSMCs and HUVECs was measured and quantified via Annexin-V and PI conjugated FACS analysis. **e** Migration ability of VSMCs and HUVECs was remarkably down-regulated after transfection with Linc00299 shRNAs (sh-Linc00299-1 and sh-Linc00299-2) compared with empty vector (sh-CTR). The data represent the mean ± SD from three independent experiments. *$P < 0.05$; **$P < 0.01$; *** $P < 0.001$ ►


a
sh-CTR
sh-Linc00299-1
sh-Linc00299-2
Relative expression of Linc00299
VSMCs
HUVECs
b
VSMCs
Relative cell viability
days
c
HUVECs
d
VSMCs
sh-CTR
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sh-Linc00299-2
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% apoptotic cells
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Fold change of migrated cells

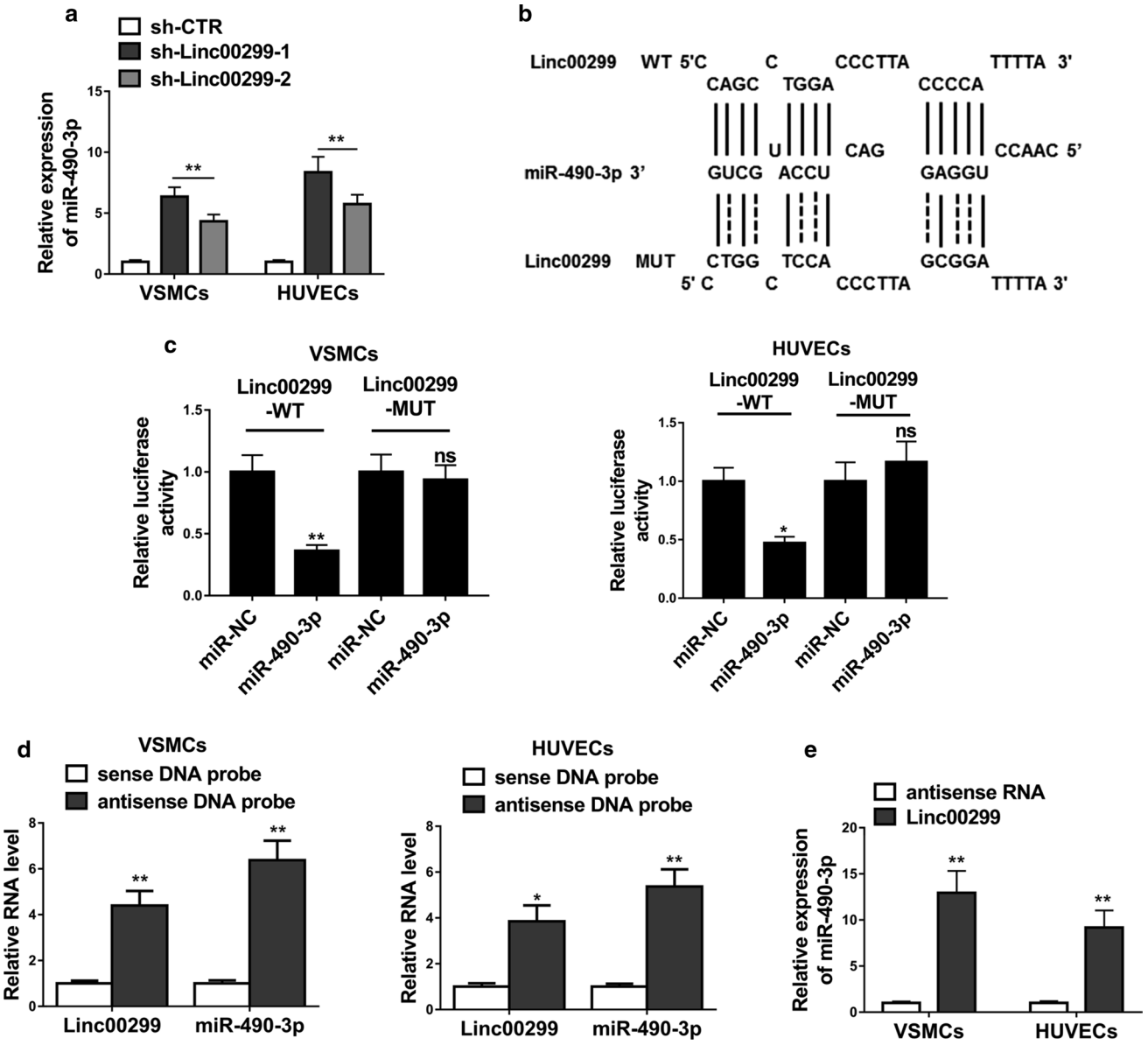


Fig. 3 Linc00299 negatively regulated and directly bound to miR-490-3p. **a** The relative expression level of miR-490-3p was significantly up-regulated in VSMCs and HUVECs after transfection with Linc00299 shRNAs (sh-Linc00299-1 and sh-Linc00299-2) compared with empty vector (sh-CTR). **b** Linc00299 segments containing the wild-type (Linc00299-WT) or mutated (Linc00299-MUT) miR-490-3p-binding sequence was displayed. **c** Luciferase reporter assay was performed in VSMCs and HUVECs cotransfected with Linc00299-WT or Linc00299-MUT and miR-490-3p or miR-NC. **d** Lysates from VSMCs and HUVECs were incubated with in vitro-synthesized biotin-labeled sense or antisense DNA probes against Linc00299 for biotin pull-down assay, followed by real-time RT–PCR analysis to examine Linc00299 and miR-490-3p levels. **e** Lysates from VSMCs and HUVECs were incubated with in vitro-synthesized biotin-labeled Linc00299 and antisense RNA for biotin pull-down assay, followed by real-time RT–PCR analysis to examine miR-490-3p levels. The data represent the mean ± SD from three independent experiments. $^{*}P < 0.05$; $^{**}P < 0.01$

(Fig. 3a). Linc00299 had a putative miR-490-3p targeting site, as predicted using miRcode (https://www.mircode.org/mircode/, Fig. 3b). Luciferase reporters carrying Linc00299-WT or Linc00299-MUT were constructed and then co-transfected with miR-490-3p or miR-NC mimics into VSMCs and HUVECs. As shown in Fig. 3c, we found that miR-490-3p could suppress the luciferase activity of Linc00299-WT, but not Linc00299-MUT, in both cells, suggesting a direct binding between miR-490-3p and Linc00299. To further investigate whether Linc00299 can directly bind to miR-490-3p, biotin pull-down assay was performed. MiR-490-3p was successfully pulled down by either biotin-labeled antisense DNA probe-enriched

endogenous Linc00299 or in vitro-synthesized biotin-labeled Linc00299 (Fig. 3d, e).

MiR-490-3p suppressed proliferation and migration and promoted apoptosis in VSMCs and HUVECs by targeting Aurora kinase A (AURKA)

We overexpressed miR-490-3p in VSMCs and HUVECs (Fig. 4a). Overexpression of miR-490-3p markedly inhibited the proliferation of both cells for at least 5 days after transfection (Fig. 4b, c). Apoptosis was enhanced by overexpression of miR-490-3p in VSMCs and HUVECs (Fig. 4d). Furthermore, overexpression of miR-490-3p robustly suppressed the migration ability of both cells (Fig. 4e). To reveal the potential mechanism underlying how miR-490-3p regulates proliferation, apoptosis and migration, we used Targetscan (https://www.targetscan.org/vert_71/) and found that binding sequences of miR-490-3p were identified in the 3′UTR of AURKA (Fig. 4f). Luciferase reporter assay was conducted to further confirm that miR-490-3p could target AURKA directly in both VSMCs and HUVECs (Fig. 4g, h). As detected by real-time PCR and Western blotting, the mRNA and protein levels of AURKA in both cells were significantly reduced and increased by miR-490-3p mimics and inhibitors, respectively (Fig. 4i, j).

Linc00299-induced dysfunctions of VSMCs are dependent on miR-490-3p

In Linc00299-overexpressed VSMCs, the mRNA and protein levels of AURKA were upregulated, but downregulated in Linc00299-overexpressed VSMCs cotransfected with miR-490-3p mimics (Fig. 5a, b). In addition, miR-490-3p significantly abolished the increased proliferation induced by Linc00299 overexpression in VSMCs (Fig. 5c). MiR-490-3p also robustly reversed the inhibitory effects of Linc00299 overexpression on VSMCs apoptosis (Fig. 5d). Furthermore, the increased migration ability of Linc00299-overexpressed VSMCs was markedly reversed by miR-490-3p (Fig. 5e).

Discussion

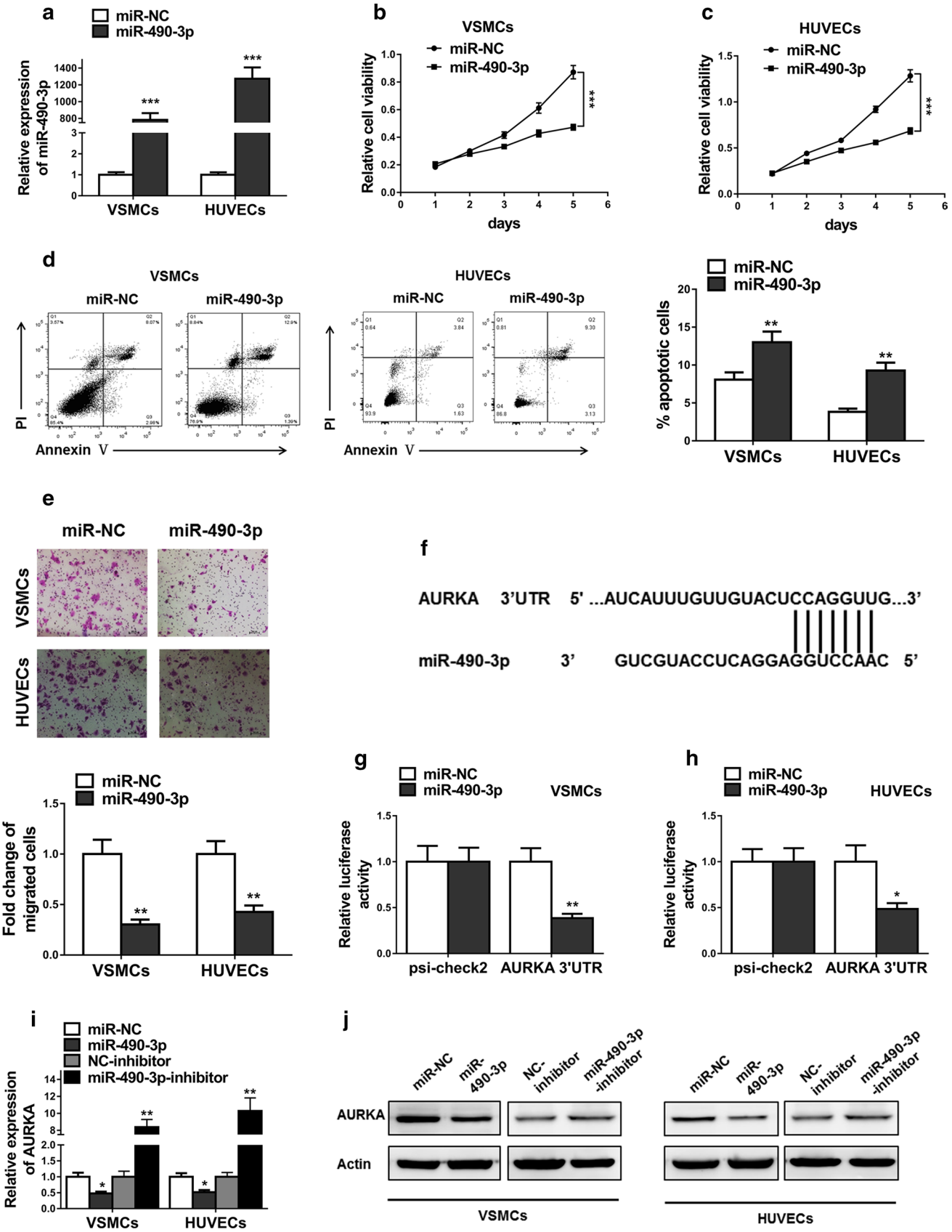
LncRNAs have emerged as important players participating in multiple biological processes, such as cell proliferation, apoptosis and migration [18]. However, the importance of lncRNAs in human diseases remains to be defined. Atherosclerosis remains to be the major cause of mortality in the world. Accumulating evidence has indicated that lncRNAs participate closely in the pathophysiological progress of atherosclerosis [11, 12]. In the present study, we investigated the effect of lncRNA Linc00299, which was upregulated in atherosclerosis patients, in regulating proliferation, apoptosis and migration of VSMCs and HUVECs and its potential targets.

Linc00299 is widely expressed in human organs and most abundantly in the brain [13]. Disruption of Linc00299 has been found in a patient of brain developmental delay, suggesting its significant role in human developmental disorders [13]. Disruption of Linc00299 is also likely to be the cause for neurocognitive disorder of a patient with balanced chromosome abnormalities [19]. The role of Linc00299 remains to be investigated in atherosclerosis. In this study, we found that the transcription of Linc00299 was significantly increased in serum and atherosclerosis plaques from atherosclerosis patients compared with that in serum and arteries from healthy controls. In addition, knockdown of Linc00299 by shRNAs markedly suppressed cell proliferation, increased apoptosis and reduced migration in VSMCs and HUVECs. Our findings suggest that Linc00299 may act as a key player in regulation of the excessive proliferation and improper migration of VSMCs and endothelial cells in atherosclerosis.

LncRNAs usually bind to certain miRNAs and act as "decoys" or "sponges" to attenuate the transcription of targeted miRNAs [20]. MiR-490-3p was proposed to be negatively regulated by Linc00299 as predicted using miRcode (https://www.mircode.org/mircode/). As expected, miR-490-3p transcription levels were significantly reduced in serum and plaques of atherosclerosis patients compared with those of healthy controls, and these changes were opposite to those in Linc00299 levels. We found that knockdown of Linc00299 by shRNAs induced upregulation of miR-490-3p in VSMCs and HUVECs. Furthermore, results from luciferase reporter assay and biotin RNA pull-down assay suggested a direct binding between Linc00299 and miR-490-3p. Echoing the changes induced by Linc00299 knockdown, overexpression of miR-490-3p reduced proliferation ability, increased apoptosis rate and suppressed migration ability of VSMCs and HUVECs. These results strongly suggested that Linc00299 may act as an endogenous sponge of miR-490-3p in VSMCs and HUVECs.

As a result of the miRNA "sponge", lncRNA positively regulates protein expression. We used Targetscan (https://www.targetscan.org/vert_71/) and identified AURKA as a potential target under regulation of miR-490-3p. The direct binding between miR-490-3p and AURKA was further validated using luciferase reporter assay. In addition, miR-490-3p mimics downregulated AURKA, whereas miR-490-3p inhibitors upregulated AURKA at both mRNA and protein levels in VSMCs and HUVECs. Therefore, Linc00299 may regulate AURKA expression via antagonizing miR-490-3p function in VSMCs and HUVECs.

Aurora kinase is a family of serine/threonine kinases and is widely distributed in multicellular organisms and is essential for mitosis and cytokinesis. AURKA is a member of


a
miR-NC
miR-490-3p
Relative expression of miR-490-3p
VSMCs
HUVECs
b
VSMCs
miR-NC
miR-490-3p
Relative cell viability
days
c
HUVECs
miR-NC
miR-490-3p
Relative cell viability
days
d
VSMCs
HUVECs
miR-NC
miR-490-3p
PI
Annexin V
% apoptotic cells
e
miR-NC
miR-490-3p
VSMCs
HUVECs
Fold change of migrated cells
f
AURKA 3'UTR 5' ...AUCAUUUGUUGUACUCCAGGUUG...3'
miR-490-3p 3' GUCGUACCUCAGGAGGUCCAAC 5'
g
miR-NC
miR-490-3p
VSMCs
Relative luciferase activity
psi-check2
AURKA 3'UTR
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miR-NC
miR-490-3p
HUVECs
Relative luciferase activity
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miR-490-3p-inhibitor
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j
miR-NC
miR-490-3p
NC-inhibitor
miR-490-3p-inhibitor
AURKA
Actin
VSMCs
HUVECs

◂**Fig. 4** MiR-490-3p suppressed proliferation and migration of VSMCs and HUVECs by targeting AURKA. **a** The relative expression levels of miR-490-3p in VSMCs and HUVECs transfected with miR-490-3p mimics or microRNA negative control (miR-NC) detected by real-time PCR. **b**, **c** Proliferation ability of VSMCs and HUVECs was significantly suppressed after transfection with miR-490-3p mimics compared with microRNA negative control (miR-NC). **d** Overexpression of miR-490-3p significantly promoted apoptosis. Apoptosis of VSMCs and HUVECs was measured and quantified via Annexin-V and PI conjugated FACS analysis. **e** Migration ability of VSMCs and HUVECs was remarkably down-regulated after transfection with miR-490-3p mimics compared with microRNA negative control (miR-NC). **f** Schematic of predicted miR-490-3p binding sites in the 3′UTR of AURKA. **g**, **h** The luciferase activities in VSMCs and HUVECs co-transfected with miR-490-3p mimics and luciferase reporters containing nothing or AURKA 3′UTR were detected as the relative ratio of hRluc luciferase activity to hluc+ luciferase activity. **i**, **j** The relative expression levels of AURKA in VSMCs and HUVECs transfected with indicated microRNA mimics or microRNA inhibitors detected by realtime-PCR and western blot. The data represent the mean ± SD from three independent experiments. *$P<0.05$; **$P<0.01$; *** $P<0.001$

the Aurora kinase. There are three different kinds of Aurora kinases in mammalian cells [21]. All Aurora kinases are found to be overexpressed in various kinds of cancers. Therefore, the Aurora kinases are the priority pharmaceutical targets for cancer treatment [22, 23].

In addition to cell cycle and proliferation, AURKA is also closely involved in the control of cell migration [24]. It has been shown that activation of AURKA promotes cell proliferation and migration through activating the cyclin E/CDK2/cyclin B1 and p38/AKT/MMP-2 pathways, respectively [24]. Noncoding RNAs, such as miR-124-3p, have been shown to regulate cancer cell proliferation, apoptosis and migration through targeting AURKA [25]. Excessive proliferation and improper migration in VSMCs and endothelial cells are key processes involved in atherosclerosis, thus AURKA inhibition is likely to be a therapeutic strategy for atherosclerosis treatment.

In the present study, we found that overexpression of Linc00299 in VSMCs significantly increased AURKA mRNA and protein levels, whereas Linc00299-induced upregulation of AURKA was abolished by miR-490-3p. As a result, Linc00299-induced increased proliferation and reduced apoptosis, and enhanced migration of VSMCs was partially suppressed by miR-490-3p. Altogether, the present results have suggested that Linc00299/miR-490-3p/AURKA axis plays a crucial role in inducing excessive

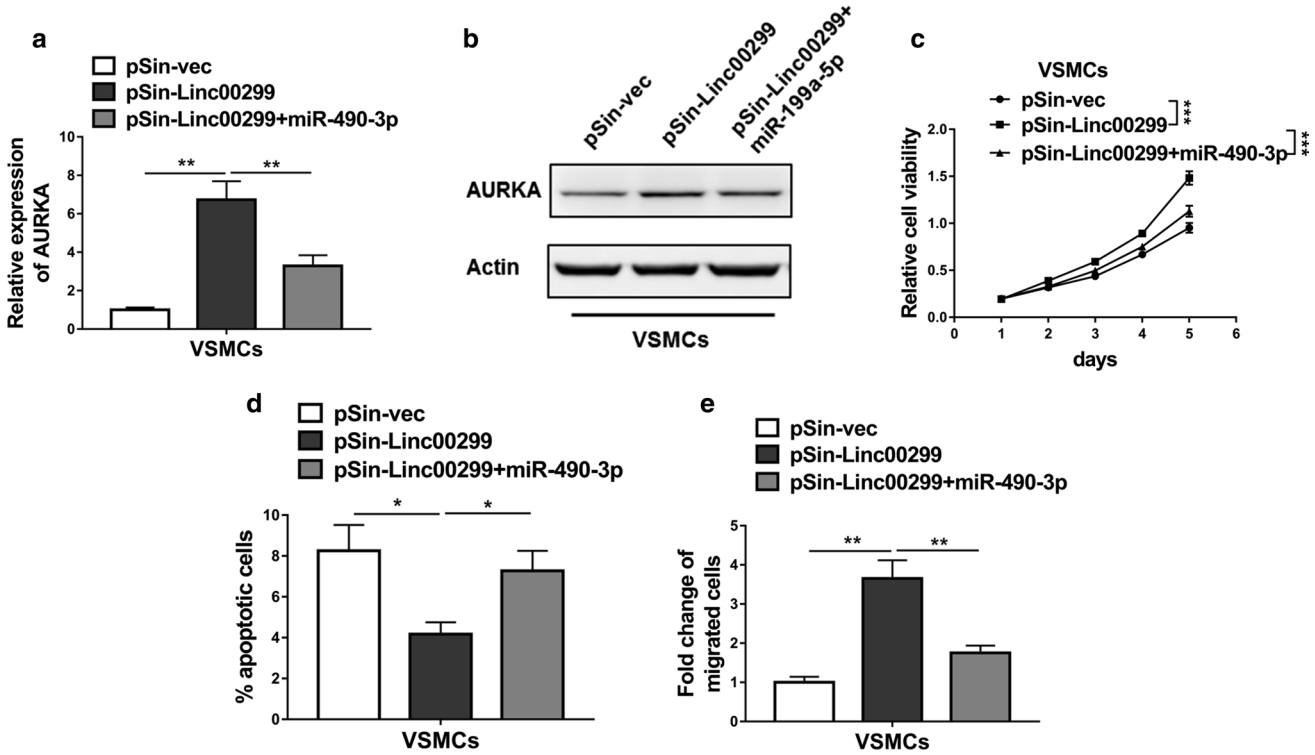
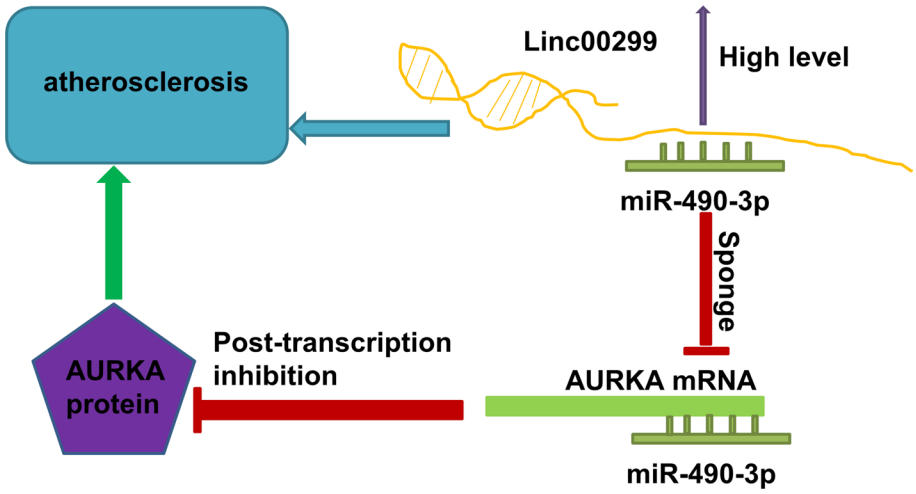


Fig. 5 Linc00299 induced dysfunctions of VSMCs was dependent on miR-490-3p. **a**, **b** The relative mRNA and protein levels of AURKA in VSMCs transfected with pSin-vec, pSin-Linc00299, or pSin-Linc00299 plus miR-490-3p mimics were detected by realtime-PCR and western blot. **c** MiR-490-3p significantly inhibited the proliferation advantage induced by overexpression of Linc00299 in VSMCs. **d** MiR-490-3p partly reverted the lower apoptosis level induced by overexpression of Linc00299 in VSMCs. **e** MiR-490-3p significantly inhibited the migration advantage induced by overexpression of Linc00299 in VSMCs. *$P<0.05$; **$P<0.01$; *** $P<0.001$



Scheme 1 A schematic illustration of the proposed model depicting a role of Linc00299/miR-490-3p/AURKA axis in atherosclerosis

proliferation and migration of VSMCs and endothelial cells in atherosclerosis.

Conclusion

The present study firstly revealed that lncRNA Linc00299 and miR-490-3p were dysregulated in atherosclerosis. Either upregulation of Linc00299 or downregulation of miR-490-3p in VSMCs and HUVECs increased cell proliferation and migration abilities. We then confirmed the direct bindings between Linc00299 and miR-490-3p, and between miR-490-3p and AURKA (see Scheme 1). Furthermore, we proved that Linc00299/miR-490-3p/AURKA axis may induce excessive proliferation and migration in VSMCs and HUVECs. Our data provide insight into the function of the Linc00299/miR-490-3p/AURKA axis in dysregulated cellular processes of VSMCs and endothelial cells in atherosclerosis.

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Compliance with ethical standards

Conflicts of interest All authors disclose that they do not have any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations and grants or other funding. All the authors state that they have no conflicts of interest.

Human and animal rights The study was approved by the Ethics Committee of Affiliated Hospital of Hebei University.

Informed consent Written informed consent had been signed by all patients.

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