Original Paper

Silence of lncRNA UCA1 Represses the Growth and Tube Formation of Human Microvascular Endothelial Cells Through miR-195

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Key Words

Atherosclerosis • Angiogenesis • lncRNA UCA1 • miR-195 • CCND1

Abstract

Background/Aims: Recent studies have suggested that several lncRNAs contribute to the angiogenic function of endothelial cells. Herein, we set out to reveal whether lncRNA UCA1 has functions in endothelial angiogenesis. **Methods:** The expression levels of lncRNA UCA1, miR-195 and CCND1 in human microvascular endothelial HMEC-1 cells were altered by transfection. Subsequently, cell viability, migration, tube formation and apoptosis of HMEC-1 cells were respectively assessed. The cross-talk between lncRNA UCA1, miR-195, CCND1, and MEK/ERK and mTOR signaling pathways were investigated by performing qRT-PCR and Western blotting. **Results:** Silence of lncRNA UCA1 repressed HMEC-1 cells viability, migration and tube formation, and induced apoptosis. Meanwhile, silence of lncRNA UCA1 significantly up-regulated miR-195 expression. These alterations induced by lncRNA UCA1 were further enhanced by miR-195 overexpression, while were attenuated by miR-195 suppression. Moreover, silence of lncRNA UCA1 deactivated MEK/ERK and mTOR signaling pathways via miR-195-dependent regulation. And the deactivation of MEK/ERK and mTOR signaling pathways led to a down-regulation of CCND1. **Conclusion:** This study demonstrates that silence of lncRNA UCA1 largely represses microvascular endothelial cells growth and tube formation. Silence of lncRNA UCA1 exerts its function possibly via up-regulation of miR-195, which in turn inactivates MEK/ERK and mTOR signaling pathways, and ultimately represses CCND1 expression.

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Introduction

Atherosclerosis is a consequence of coronary artery disease, stroke, and peripheral artery disease. If not treated promptly, atherosclerosis can cause the ischemia and necrosis of limbs, and eventually result in amputation. Current therapies prevent vein graft atherosclerosis and are essentially limited to lipid-lowering drugs, typically statins [1], fibrates [2], niacin [3], and ezetimibe [4]. Lipid-lowering drugs decrease low-density lipoprotein cholesterol (LDL-C) level and reduce the risk of atherosclerotic cardiovascular diseases. However, many high-risk people have levels of LDL-C that remain unacceptably high despite being treated with maximally tolerated doses of lipid-lowering drugs [5]. Thus, a better understanding of atherosclerosis is still required to improve the therapeutic strategy of atherosclerosis.

Non-coding RNAs (ncRNAs) cover more than 98% of the human genome [6]. Among them, microRNAs (miRNAs) have been extensively studied, while little is known about the role of long ncRNAs (lncRNAs). Recent studies have partially revealed the regulatory functions of lncRNAs in complex human diseases, such as a variety of cancers [7], neurological disorder [8] and atherosclerosis [9]. Further investigations indicate that lncRNAs may regulate the biological processes of atherosclerosis-related endothelial cells, especially angiogenesis. For the selected examples, it has been reported that silence of lncRNA H19 resulted in age-induced impairment of angiogenesis [10]. LncRNA SENCER has been shown to be significantly up-regulated in endothelial derivatives of human embryonic stem cells, and it controlled the sprouting and migratory capacity of endothelial cells [11].

Urothelial carcinoma associated 1 (UCA1), a lncRNA initially discovered in bladder cancer [12], which has been reported to be an oncogene in many cancers, including bladder cancer [13], gastric cancer [14], oral squamous cell carcinoma [15], and prostate cancer [16]. Recent literatures have mainly focused on the expression pattern and the function of lncRNA UCA1 in various cancers, and the potential regulatory role of lncRNA UCA1 in cancer cells proliferation, invasion, migration and apoptosis has been widely reported [13, 14, 17]. Besides, although the underlying mechanisms of which lncRNA UCA1 functioned to malignant growth and metastasis are still unclear, bioinformatics analyses have suggested that it might be by sponging miRNAs and thereby quarantining the target gene of miRNAs from degradation [15].

To date, little is known regarding the exact role of lncRNA UCA1 in atherosclerosis. This study concerned about this issue by investigating the functional effect of lncRNA UCA1 silence on human microvascular endothelial HMEC-1 cells growth and tube formation. Additionally, the interaction between lncRNA UCA1 and miR-195 was explored to identify whether miR-195 (a microvascular-associated miRNA [18, 19]) was involved in the action of lncRNA UCA1.

Materials and Methods

Cell Culture

Human microvascular endothelial HMEC-1 cells purchased from American Type Culture Collection (Cat No: CRL-3243; ATCC, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle's Medium (Gibco, Sigma-Aldrich, St. Louis, MO, USA). Heat-inactivated fetal bovine serum (Life Technologies, Cergy Pontoise, France) was supplemented into the culture medium with a final concentration of 10% (v/v). The cells were maintained at 37°C in a humidified 5% CO₂ incubator. Third to seventh passages were used for angiogenesis assay. Third to tenth passages were used for other assays.

For the inhibition of MEK and mTOR pathways, cells were respectively treated with 10 nM AZD6244 (Selleck Chemicals, Houston, TX, USA) and 20 μM Tamoxifen (Sigma-Aldrich, St. Louis, MO, USA) for 24 h.

Transfection

For lncRNA UCA1 silencing, shRNA anti-human lncRNA UCA1 was ligated into the pGPU6/Neo plasmid (GenePharma, Shanghai, China). A non-targeting sequence was used as a blank control. For miR-195 overexpression and suppression, the specific mimic and inhibitor for human miR-195 were transfected into cell. The synthesized sequence of cel-miR-67-3p was transfected as a blank control. The full-length wild-type of CCND1 was cloned into a pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA) to make a CCND1 expression vector. An empty pcDNA3.1 vector was transfected as a blank control. CCND1 shRNA plasmid (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was transfected in order to silence the expression of CCND1. A non-targeting sequence was used as a blank control. All transfection was performed by using Lipofectamine 3000 reagent (Life Technologies Corporation, Carlsbad, CA, USA). At 48 h of transfection, cells were collected and the transfection efficiency was verified by qRT-PCR or Western blot analysis.

qRT-PCR

Total RNA was extracted from cell by using TRI Reagent (Sigma-Aldrich). The purity and concentration of the extract RNA were tested by SMA 400-UV-VIS (Merinton, Shanghai, China). For the test of EGFR2, CD144, eNOS, lncRNA UCA1, and CCND1, the PrimerScript 1st Strand cDNA Synthesis Kit (Invitrogen) was used for cDNA synthesis, and the SYBR ExScript RT-qPCR Kit (Takara, China) was used for qRT-PCR. β -actin was used as an internal control in this procedure. For the miR-195, the Taqman MicroRNA Reverse Transcription Kit and Taqman Universal Master Mix II (both from Applied Biosystems, Foster City, CA, USA) were used for cDNA synthesis and qRT-PCR respectively. U6 was used as an internal control. Data were calculated by relative quantification ($2^{-\Delta\Delta Ct}$) method.

Cell viability assessment

The viability of transfected cells was detected by using Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Kyushu, Japan). The transfected cells were planted into 96-well plates with a density of 5000 cells/well. After 48 h of incubation, the culture medium was removed and 10 μ l CCK8 was added into each well. Following 4 h of incubation at 37°C, the absorbance of each well was measured by a Bio-Rad Microplate Reader 550 (Bio-Rad, Hercules, CA, USA) at 490 nm.

Quantitation of apoptosis

The apoptosis of the transfected cells was tested by using an Annexin V-FITC/PI Apoptosis Detection Kit (Qcbio Science & Technologies Co., Ltd., Shanghai, China). Cells (1×10^5) were resuspended in 100 μ l Binding Buffer containing 5 μ l Annexin V-FITC and 10 μ l PI Staining Solution. The samples were incubated at room temperature for 15 min in the dark, after which 400 μ l Binding Buffer was added. Flow cytometry analysis was done on a FACS can (FACS Calibur, Beckman Coulter, Fullerton, CA, USA). The percentage of apoptotic cells (Annexin V-FITC positive and PI negative) was calculated by FlowJo software version 7.6.4 (Tree Star, San Carlos, California, USA).

Transwell assay

Transwell assay was performed to detect cell migration by using a modified Boyden chamber (Costar, Corning, New York, USA). The transfected cells (1×10^5) were placed in the upper chamber with serum-free medium. The lower chamber was filled with 200 μ l complete medium. The cells were incubated at 37°C for 24 h, after which the non-traversed cells in the upper side were removed by cotton swabs, and the cells on the lower side were stained with crystal violet (Beyotime, Nantong, China) for 30 min. The stained cells were then counted under an optical microscope (BX51 system, Olympus, Tokyo, Japan).

In vitro angiogenesis assay

For angiogenesis assay, 6-well plates were pre-coated with matrigel (BD Biosciences, San Jose, CA, USA). Then, 1×10^5 HMEC-1 cells were seeded into each well. After 24 h of culturing at 37°C, the formation of capillary-like structures was photographed and the number of capillary-like structures was counted. The tube length was analyzed by the AxioVision Rel software version 4.8 (Carl Zeiss AG, Jena, Germany) [20].

Western blot analysis

Cellular protein was isolated by using RIPA lysis buffer (Beyotime, Shanghai, China). The purity and concentration of the extract protein were verified by the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Protein (0.1 mg) from each sample was resolved over SDS-PAGE and transferred onto a polyvinylidene fluoride membrane. The membranes were blocked in 5% non-fat milk for 1 h at room temperature, and then incubated at 4°C overnight with primary antibodies: anti-VEGF (5365-100, BioVision, Milpitas, CA, USA), anti-VEGFR2 (OAEC00429, Aviva Systems Biology, San Diego, CA, USA), anti-CD144 (sc-52751), anti-Bcl-2 (sc-509), anti-Bax (sc-6236), anti-caspase-3 (sc-271028), anti-caspase-9 (sc-133109), anti-CCND1 (sc-450), anti-β-actin (sc-130300, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-eNOS (ab76210), anti-t-MEK (ab32091), anti-p-MEK (ab96379), anti-t-ERK (ab32537), anti-p-ERK (ab13143), anti-t-mTOR (ab2732), anti-p-mTOR (ab109268), anti-t-S6K (ab32529), anti-p-S6K (ab2571, Abcam, Cambridge, MA, USA). The membranes were then incubated with the secondary antibodies for 1 h at room temperature, and the signals were visualized by enhanced chemiluminescence method. The intensity of the bands was quantified using Image Lab™ Software version 5.0 (Bio-Rad, Laboratories, Hercules, CA, USA).

Statistics

Excepted for Western blot analysis, data are given as means ± SD. Statistical analysis was performed by using the SPSS version 19.0 program (SPSS Inc., Chicago, IL, USA). Statistical differences between two or more groups were analyzed by using a one-way analysis of variance (ANOVA) with Duncan post-hoc. Before ANOVA, all data were tested for normal distribution and similar variance. *P* values of <0.05 was considered as significant.

Results

Tube formation of HMEC-1 cells

HMEC-1 cells were grown in microtiterated plates for 24h, and the formation of capillary-like tubes was monitored. As shown in Fig. 1A-1B, the length and rate of capillary-like tubes were increased significantly with the prolongation of time (*P* < 0.05). In addition, the mRNA and protein levels of VEGF, VEGFR2, CD144 and eNOS were all significantly increased in a time-dependent manner (Fig. 1C-1D, *P* < 0.05). Compared to other time points, 24 h of culturing achieved the greatest tube formation and highest protein levels of angiogenesis-related factors. Thus, 24 h was selected as a culturing condition for use in the following experiments.

LncRNA UCA1 down-regulated HMEC-1 cells growth and tube formation capacity

To test the potential role of lncRNA UCA1 in the angiogenesis of endothelial cells, the expression of lncRNA UCA1 in HMEC-1 cells was silenced by shRNA transfection (*P* < 0.05, Fig. 2A). Silence of lncRNA UCA1 by shRNA-based knockdown significantly decreased cell viability, migration, and tube formation (*P* < 0.05, Fig. 2B-2E), while significantly increased the apoptotic cell rate (*P* < 0.05, Fig. 2F). Besides, silence of lncRNA UCA1 down-regulated the protein expression of Bcl-2, up-regulated the protein expression of Bax, and remarkably increased caspase-3 and caspase-9 (Fig. 2G).

LncRNA UCA1 silence up-regulated the expression of miR-195

A previous study has revealed that lncRNA UCA1 was a molecular sponge for miR-195 in bladder cancer cells [13]. Herein, we studied whether lncRNA UCA1 functioned to HMEC-1 cells also in a miR-195-dependent regulation. By performing qRT-PCR we found that the RNA level expression of miR-195 was significantly increased by genetic deletion of lncRNA UCA1 (*P* < 0.05, Fig. 3), indicating miR-195 also can be negatively regulated by lncRNA UCA1 in HMEC-1 cells.

Fig. 1. Tube formation of HMEC-1 cells. HMEC-1 cells were grown on matrigel-coated plates for 1, 6, 12 and 24 h. (A) The length and (B) rate of capillary-like tubes. (C) mRNA levels of VEGF, VEGFR2, CD144, and eNOS. (D) Protein levels of VEGF, VEGFR2, CD144, and eNOS. Data presented as mean $\pm$ SD (n = 3). Different letters above the columns in the same culture time indicate that the means of different groups were significantly different (ANOVA with Duncan post hoc test).

LncRNA UCA1 silence represses HMEC-1 cells growth and tube formation via up-regulation of miR-195

To establish a link between miR-195 and endothelial angiogenic capacity, the mimic and inhibitor specific for miR-195 were transfected into HMEC-1 cells. As shown in Fig. 4A, the expression of miR-195 was significantly increased by mimic transfection, while was significantly reduced by inhibitor transfection ($P < 0.05$). The decreases in viability, migration, and tube formation induced by lncRNA UCA1 silence were significantly accelerated by miR-195 mimic, while were significantly attenuated by miR-195 inhibitor ($P < 0.05$, Fig. 4B-4E). Also, lncRNA UCA1 silence induced apoptosis was accelerated by miR-195 mimic, and was significantly attenuated by miR-195 inhibitor, as evidenced by the changes in apoptotic cell rate and protein levels of apoptosis-related factors ($P < 0.05$, Fig. 4F-4G).

LncRNA UCA1 silence deactivated MEK/ERK and mTOR signaling pathways via up-regulation of miR-195

To get insights into the molecular mechanism by which lncRNA UCA1 controls endothelial cells function, we tested the activation of MEK/ERK and mTOR signaling pathways. Data in Fig. 5A and 5B demonstrated that, phosphorylation forms of MEK, ERK, mTOR and S6K were all remarkably down-regulated, while the total levels of these proteins were unchanged in response to lncRNA UCA1 silence. As compared to the sh-NC+NC group, the ratio of phospho/total (p/t)-MEK, p/t-ERK, p/t-mTOR and p/t-S6K were all significantly decreased in sh-UCA1+NC group ($P < 0.05$), indicating lncRNA UCA1 silence repressed the activation of MEK/ERK and mTOR signaling. Moreover, we found that the deactivation induced by lncRNA UCA1 silence was further enhanced by miR-195 mimic, while was recovered by miR-195 inhibitor ($P < 0.05$).

Fig. 2. Effect of lncRNA UCA1 silence on HMEC-1 cells growth and tube formation. HMEC-1 cells were transfected with a shRNA against lncRNA UCA1 (sh-UCA1) or a negative control (shNC). (A) RNA levels of lncRNA UCA1. (B) Cell viability of transfected cells. (C) Relative migration of transfected cells. (D) The length and (E) rate of capillary-like tubes after transfection. (F) Apoptotic cell rate of transfected cells. (G) Protein levels of apoptosis-related factors. Data presented as mean $\pm$ SD (n = 3). *, P<0.05 (ANOVA).

Fig. 3. Effect of lncRNA UCA1 silence on miR-195 expression. RNA levels of miR-195 in HMEC-1 cells transfected with a shRNA against lncRNA UCA1 (sh-UCA1) or a negative control (shNC). Data presented as mean $\pm$ SD (n = 3). *, P<0.05 (ANOVA).

Fig. 4. Effect of miR-195 on lncRNA UCA1-mediated cell growth and tube formation. (A) RNA levels of miR-195 in HMEC-1 cells which were transfected with miR-195 mimic, miR-195 inhibitor or a negative control (NC). (B) Cell viability, (C) relative migration, (D) the length and (E) rate of capillary-like tubes, (F) apoptotic cell rate, and (G) protein levels of apoptosis-related factors, in cell after co-transfection with sh-UCA1 and miR-195 mimic/inhibitor/NC. Data presented as mean $\pm$ SD (n = 3). *, P<0.05 (ANOVA).

Fig. 5. Effect of lncRNA UCA1 silence and miR-195 dysregulation on the activation of MEK/ERK and mTOR signaling pathways. (A) Protein levels of MEK, ERK, and (B) mTOR, S6K in cell after co-transfection with sh-UCA1 and miR-195 mimic/inhibitor/NC. Data presented as mean $\pm$ SD (n = 3). *, P < 0.05 (ANOVA).

Fig. 6. Effect of blocking MEK/ERK and mTOR signaling pathways on the expression of CCND1. (A) Protein levels of MEK, ERK, and (B) mTOR, S6K in cell after co-transfection with sh-UCA1 and miR-195 inhibitor. AZD6244 and Tamoxifen were added into culture medium to inhibit MEK and mTOR pathways, respectively.

Fig. 7. Effect of CCND1 dysregulation on HMEC-1 cells growth and tube formation. HMEC-1 cells were transfected with CCND1 expression vector (pc-CCND1), shRNA against CCND1 (sh-CCND1) or the negative controls (pcDNA and shNC). (A) Protein and mRNA levels of CCND1. (B) Cell viability of transfected cells. (C) Relative migration of transfected cells. (D) The length and (E) rate of capillary-like tubes after transfection. (F) Apoptotic cell rate of transfected cells. (G) Protein levels of apoptosis-related factors. Data presented as mean $\pm$ SD (n = 3). *, P < 0.05 (ANOVA).

LncRNA UCA1 silence decreased the expression of CCND1

The deactivation of MEK/ERK and mTOR signaling induced by lncRNA UCA1 silence was further studied by using two inhibitors of MEK and mTOR, i.e., AZD6244 and Tamoxifen. Results showed that the elevated levels of p-MEK and p-ERK were both suppressed by AZD6244 treatment (Fig. 6A). Also, the elevated levels of p-mTOR and p-S6K were both suppressed by Tamoxifen treatment (Fig. 6B). Additionally, the protein expression of CCND1 was decreased by sh-UCA1, and was increased by miR-195 inhibitor. More interestingly, AZD6244 and Tamoxifen flattened the increase of CCND1 levels induced by miR-195 inhibitor.

CCND1 promoted HMEC-1 cells growth and tube formation

To address the question whether CCND1 contributed to endothelial cells angiogenesis, the expression level of CCND1 was overexpressed or suppressed by transfection. The mRNA and protein levels of CCND1 were significantly increased by pc-CCND1 transfection, and were decreased by sh-CCND1 transfection ($P < 0.05$, Fig. 7A). As expected, cell viability, migration, and tube formation were significantly increased by pc-CCND1, and were significantly reduced by sh-CCND1 ($P < 0.05$, Fig. 7B-7E). Apoptosis was not reduced by pc-CCND1, perhaps because the apoptosis in control group was already pretty low; but apoptosis was significantly induced by sh-CCND1 ($P < 0.05$, Fig. 7G).

Discussion

Plaque angiogenesis is one of the important contributors to unstable lesions [21]. It can cause occlusive thrombosis and finally cause a variety of acute cardiovascular events [22]. In angiogenesis-driving processes, endothelial cells proliferation, migration and invasion are involved in angiogenic sprouting [23]. Besides, endothelial cells proliferation and tube formation are driven by VEGF and its receptors (VEGFR1 and VEGFR2) [24]. In addition to VEGF, CD144 and eNOS are two other important players in angiogenesis. eNOS mobilizes endothelial progenitor cells from bone marrow to peripheral blood, and causes ischemia-induced vascularization [25]. The dynamics of CD144 is required for angiogenic endothelial cells migration and positional rearrangement [26]. In this study, we investigated the role and mechanism of lncRNA UCA1 in human microvascular endothelial HMEC-1 cells growth and tube formation. We demonstrated that silence of lncRNA UCA1 repressed HMEC-1 cells viability, migration, tube formation, and accelerated a mitochondria initiated apoptosis. Meanwhile, miR-195 was highly expressed by lncRNA UCA1 silence. Rescue experiments identified that lncRNA UCA1 conferred its function might be partially via regulating miR-195 expression. We further found that miR-195 may inactivate MEK/ERK and mTOR signaling pathway, which may then repress CCND1 expression.

The functional role of lncRNA UCA1 in endothelial cells angiogenesis is largely unknown at present. However, a previous study has reported that lncRNA UCA1 was up-regulated in hyperplastic endothelial cells of type 2 diabetic patients, demonstrating an important role of lncRNA UCA1 in lymphatic endothelial cells [27]. This was confirmed by a study of Churaman *et al.*, in which knockdown of lncRNA UCA1 has been pointed out to induce a reduction of proliferation and migration in endothelial cells [28]. In the current study, lncRNA UCA1 knockdown significantly repressed HMEC-1 cells viability, migration and tube formation, and significantly induced apoptosis. This finding suggested that lncRNA UCA1 might be one of the pro-angiogenic factors in atherosclerosis, and provided *in vitro* evidences that lncRNA-related strategies will be beneficial in patients with atherosclerosis.

It has been widely reported that miR-195 exerted inhibitory effects on angiogenesis in a wide range of cancers, such as hepatocellular carcinoma [29], prostate cancer [30] and breast cancer [31]. miR-195 inhibited the expression of pro-angiogenic factor VEGF and the pro-metastatic factors VAV2 and CDC42 [29]. More interestingly, miR-195 was highly expressed in a specific endothelium which is strongly positive for CD31 and endomucin (CD31^{hi} Emcn^{hi}) [32]. Besides, miR-195 inhibition promoted human endothelial progenitor cells

proliferation, migration and angiogenesis under hypoxic condition [33]. These studies demonstrated an anti-angiogenic role of miR-195 in endothelial cells, which were consistent with the findings revealed in this study. We found that miR-195 was highly expressed in HMEC-1 cells after transfection with the shRNA specific for lncRNA UCA1, indicating lncRNA UCA1 negatively regulated miR-195 expression. Moreover, the functional impacts of lncRNA UCA1 silence on HMEC-1 cells growth, migration and tube formation were attenuated when miR-195 was suppressed. Based on these findings we preliminarily concluded that the impacts of lncRNA UCA1 silence on HMEC-1 cells growth and tube formation possibly via up-regulation of miR-195.

The activation of MEK/ERK signaling pathway is pivotal in modulating a number of cellular processes including proliferation and angiogenesis [34]. MEK activation is the upstream signaling event that leads to the activation of ERK. During atherosclerosis, ERK modulates macrophage cholesterol homeostasis and key genes implicated in atherosclerosis [35]. Besides, many researchers have suggested that mTOR may offer new avenues in the regression of atherosclerotic plaques [36, 37]. mTOR contributes in proliferation, lipogenesis and energy metabolism possibly by targeting S6K and cyclin-dependent kinases (CDKs) [38]. Previous studies have evidenced that lncRNA UCA1 promoted malignant transformation and cancer progression through activation of mTOR pathway [14, 39]. However, the relation between lncRNA UCA1 and MEK/ERK pathway has not been revealed yet. In the current study, we demonstrated that lncRNA UCA1 silence up-regulated miR-195 and inactivated MEK/ERK and mTOR signaling pathways. Additionally, we demonstrated that blockade of MEK/ERK and mTOR signaling pathways by using their corresponding inhibitors (AZD6244 and Tamoxifen) led to the down-regulation of CCND1, indicating CCND1 might be one of the executors in the revealed regulation system. Furthermore, the hypothesis was confirmed in the functional experiments, from which the pro-growth, pro-migration and pro-tube formation effects of CCND1 were observed.

This study highlighted the potential anti-angiogenic effect of lncRNA UCA1 silence on HMEC-1 cell line under normal conditions. Since other types of cells, such as macrophage, foam cells, monocytes and smooth muscle cells are also implicated in the pathogenesis of atherosclerosis, the role of lncRNA UCA1 in these cells are required to be further studied. Additionally, the regulatory impacts of lncRNA UCA1 on the release of inflammatory cytokines and lipid cholesterol are needed to be studied which will largely improve our understanding of lncRNA UCA1 in atherosclerosis.

Conclusion

In conclusion, our study demonstrates that silence of lncRNA UCA1 represses microvascular endothelial cells growth and tube formation capacity. Silence of lncRNA UCA1 exerts its function possibly via up-regulation of miR-195, which in turn inactivates MEK/ERK and mTOR signaling pathways, and ultimately represses CCND1 expression (Fig. 8). This finding provided the first evidence that lncRNA UCA1 played significant roles in endothelial dysfunction.

Fig. 8. The molecular mechanism by which lncRNA UCA1 silence represses microvascular endothelial cells growth and tube formation capacity, which is revealed in this study.

Disclosure Statement

The authors declare that they have no competing interests.

References

- Amarenco P, Labreuche J, Lavallee P, Touboul PJ: Statins in stroke prevention and carotid atherosclerosis: a systematic review and up-to-date meta-analysis. *Stroke* 2004;35:2902-2909.
- Ito MK: Long-chain omega-3 fatty acids, fibrates and niacin as therapeutic options in the treatment of hypertriglyceridemia: a review of the literature. *Atherosclerosis* 2015;242:647-656.
- Jackevicius CA, Tu JV, Ko DT, de Leon N, Krumholz HM: Use of niacin in the United States. *Ann Intern Med* 2013;173:1379-1381.
- Bogiatzi C, Spence JD: Ezetimibe and regression of carotid atherosclerosis: importance of reducing plaque burden. *Stroke* 2012;43:1153-1155.
- Barter PJ, Rye KA: New Era of Lipid-Lowering Drugs. *Pharmacol Rev* 2016;68:43-53.
- Lorenzen JM, Thum T: Long noncoding RNAs in kidney and cardiovascular diseases. *Rev Nephrol* 2016;12:360-373.
- Bolha L, Ravnik-Glavac M: Long Noncoding RNAs as Biomarkers in Cancer. *Int J Mol Sci* 2017;18:7243968.
- Roberts TC, Morris KV, Wood MJ: The role of long non-coding RNAs in development, brain function and neurological disease. *Philos Trans R Soc Lond B Biol Sci* 2014;369:20130507.
- Li H, Zhu H, Ge J: Long Noncoding RNA: Recent Updates on Atherosclerosis. *Int J Biol Sci* 2016;12:898-910.
- Boon RA, Hofmann P, Michalik KM, Lozano-Vidal N, Pahl S, D, Fischer A, Knau A, Jae N, Schurmann C, Dimmeler S: Long Noncoding RNA Meg3 Controls Endothelial Aging and Function: Implications for Regenerative Angiogenesis. *J Am Coll Cardiol* 2016;67:589-592.
- Boulberdaa M, Scott E, Ballantyne M, Garcia-Cardena G, Angelini GD, Brittan M, Hunter A, McBride M, McClure J, Miano JM, Emanueli C, Mills N, Mourad JC, Baker AH: A Role for the Long Noncoding RNA SENCER in Commitment and Function of Endothelial Cells. *Mol Ther* 2016;24:978-990.
- Wang XS, Zhang Z, Wang HC, Chen QW, Chen YC, Qian XP, Lu TJ, Yu LZ, Zhang Y, Xin DQ, Na YQ, Chen WF: Rapid identification of bladder cancer as a very sensitive and specific unique marker for human bladder carcinoma. *Clin Cancer Res* 2006;12:1550-1558.
- Li HJ, Sun XM, Li ZK, Yin J, Pang H, Pan JJ, Li X, Chen W: LncRNA UCA1 Promotes Mitochondrial Function of Bladder Cancer via the p38-195-ARL2 Signaling Pathway. *Cell Physiol Biochem* 2017;43:2548-2561.
- Li C, Liang G, Yang S, Sui J, Chen X, Zhang Y, Peng H, Hong W, Xu S, Wu W, Ye Y, Zhang Z, Zhang W, Yin L, Pu Y: Dysregulation of lncRNA-UCA1 contributes to the progression of gastric cancer through regulation of the PI3K-Akt/mTOR signaling pathway. *Oncotarget* 2017;8:93476-93491.
- Fang Z, Guo J, Zhang W, Chen Q, Wang H, Qiao B: LncRNA UCA1 promotes proliferation and cisplatin resistance of nasopharyngeal carcinoma by suppressing miR-184 expression. *Oncotarget* 2017;6:2897-2908.
- Shang Y, Zhang X, Ji T, Chen G, Shan L: Long non-coding RNA UCA1 promotes cell progression by acting as a competing endogenous RNA of ATF2 in prostate cancer. *Am J Transl Res* 2017;9:366-375.
- Li Y, Yao Y, Leng K, Li Z, Qin W, Zhong X, Kang P, Wan M, Jiang X, Cui Y: Long non-coding RNA UCA1 promotes an unfavorable prognosis and promotes tumorigenesis via regulating AKT/GSK-3beta signaling pathway in cholangiocarcinoma. *Oncotarget* 2017;8:96203-96214.
- Wang YS, Wang HY, Liao YC, Tsai PC, Chen KC, Cheng HY, Lin RT, Juo SH: MicroRNA-195 regulates vascular smooth muscle cell phenotype and prevents neointimal formation. *Cardiovasc Res* 2012;95:517-526.
- Bras JP, Silva AM, Calin GA, Barbosa MA, Santos SG, Almeida MI: miR-195 inhibits macrophages pro-inflammatory profile and impacts the crosstalk with smooth muscle cells. *Plos One* 2017;12:e0188530.
- Castro Martins M, Peffers MJ, Lee K, Rubio-Martinez LM: Effects of stanozolol on normal and IL-1beta-stimulated equine chondrocytes *in vitro*. *BMC Vet Res* 2018;14:103.
- Michel JB, Virmani R, Arbustini E, Pasterkamp G: Intravascular haemorrhages as the trigger of plaque vulnerability. *Eur Heart J* 2011;32:1977-1985, 1985a, 1985b, 1985c.
- Hansson GK, Libby P, Tabas I: Inflammation and plaque vulnerability. *J Intern Med* 2015;278:483-493.
- Parma L, Baganha F, Quax PHA, de Vries MR: Plaque angiogenesis and intraplaque hemorrhage in atherosclerosis. *Eur J Pharmacol* 2017;816:107-115.

- 24 Carmeliet P, Jain RK: Molecular mechanisms and clinical applications of angiogenesis. *Nature* 2011;473:298-307.
- 25 Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S: Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med* 2003;9:1370-1376.
- 26 Yamamoto H, Ehling M, Kato K, Kanai K, van Lessen M, Frye M, Zeuschner D, Nakayama M, Vestweber D, Adams RH: Integrin beta1 controls VE-cadherin localization and blood vessel stability. *Nat Commun* 2015;6:6429.
- 27 Qi M, Zhou Q, Zeng W, Shen M, Liu X, Luo C, Long J, Chen W, Zhang J, Yan S: Analysis of Long Non-Coding RNA Expression of Lymphatic Endothelial Cells in Response to Type 2 Diabetes. *Cell Physiol Biochem* 2017;41:466-474.
- 28 Sethuraman S, Gay LA: microRNA dependent and independent deregulation of long non-coding RNA by an oncogenic herpesvirus. 2017;13:e1006508.
- 29 Wang R, Zhao N, Li S, Fang JH, Chen MX, Yang J, Jia WH, Yuan Y, Zhuang SM: MicroRNA-195 promotes angiogenesis and metastasis of hepatocellular carcinoma by inhibiting the expression of VEGFR2, and CDC42. *Hepatology* 2013;58:642-653.
- 30 Cai C, Chen QB, Han ZD, Zhang YQ, He HC, Chen JH, Chen YR, Yang SH, Xu YD, Zeng Y, Chen GQ, Liang YX, Dai QS, Jiang FN, Wu SL, Zeng GH, Zhong WD, Wu CL: miR-195 Inhibits Cell Proliferation Progression by Targeting RPS6KB1 in Human Prostate Cancer. *Clin Cancer Res* 2015;21:4921-4934.
- 31 Wang Y, Zhang X, Zou C, Kung HF, Lin MC, Dress A, Wardle F, Jiang B: miR-195 inhibits tumor growth and angiogenesis through modulating IRS1 in breast cancer. *Biomed Pharmacother* 2016;80:95-101.
- 32 Yang M, Li CJ, Sun X, Guo Q, Xiao Y, Su T, Tu ML, Peng Y, Li Q, Liu Q, He H, Wang TJ, Lei MX, Wan M, Cao X, Luo XH: MiR-497 approximately 195 cluster regulates osteogenesis during coupling with osteogenesis by maintaining endothelial Notch and HIF-1alpha activation. *Nat Commun* 2017;8:16003.
- 33 Mo J, Zhang D, Yang R: MicroRNA-195 regulates proliferation, migration, angiogenesis and autophagy of endothelial progenitor cells by targeting GAP-43. *Cell Prolif* 2016;36.
- 34 Ferrell JE, Jr.: MAP kinases in mitogenesis and development. *Curr Top Dev Biol* 1996;33:1-60.
- 35 Li N, McLaren JE, Michael DR, Clement M, Wang C, Ramji DP: ERK is integral to the IFN-gamma-mediated activation of STAT1. *J Biol Chem* 2005;280:10341-10348.
- 36 Huang J, Manning BD: A complex interplay between Akt, TSC2 and the two mTOR complexes. *Biochem Soc Trans* 2009;37:217-222.
- 37 Lee IS, Bourantas CV, Matsu T, Gogas BD, Heo JH, Diletti R, Farooq V, Zhang Y, Onuma Y, Serruys PW, Garcia-Garcia HM: Assessment of late evolution in coronary bifurcations located beyond everolimus eluting scaffolds by intravascular ultrasound virtual histology study. *Cardiovasc Ultrasound* 2013;11:25.
- 38 Kurdi A, de Meyer GR, Verbinet W: Potential therapeutic effects of mTOR inhibition in atherosclerosis. *Br J Clin Pharmacol* 2012;126:1267-1279.
- 39 Wu C, Li M: Long Non-Coding RNA (lncRNA) Urothelial Carcinoma-Associated 1 (UCA1) Enhances Chemoresistance in Breast Cancer Cells via Inhibiting mTOR Signaling Pathway. *Med Sci Monit* 2016;22:3860-3867.