Life Sciences 226 (2019) 33–46

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Silence of long non-coding RNA UCA1 inhibits hemangioma cells growth, migration and invasion by up-regulation of miR-200c

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ARTICLE INFO

Keywords:
Infantile hemangioma
lncRNA UCA1
miR-200c
EOMA cells
HemECs cells

ABSTRACT

Background: Human urothelial carcinoma associated 1 (UCA1) has been recognized as an oncogenic lncRNA in various cancers, except infantile hemangioma (IH). This study attempts to explore the functional role of lncRNA UCA1 in IH.

Methods: qRT-PCR was carried out to detect the expression of lncRNA UCA1 in human IH tissues. Two hemangioma cell lines (EOMA and HemECs) were transfected with shRNAs specific for lncRNA UCA1, or a plasmid for expression lncRNA UCA1. The expression of miR-200c in cell was suppressed or overexpressed by miRNA-mediated transfection. CCK-8 assay, flow cytometry, Transwell assay, and Western blot were performed to detect cell survival, migration and invasion.

Results: LncRNA UCA1 was up-regulated in proliferating-phase hemangioma samples, as compared to involuting-phase. Silence of lncRNA UCA1 significantly reduced EOMA cells viability, migration and invasion, and induced apoptosis. These observations were coupled with the down-regulations of CyclinD1, CDK6 and CDK4, the cleavage of caspase-3 and caspase-9, as well as the decreased expression levels of MMP-9 and Vimentin. miR-200c was highly expressed in lncRNA UCA1 silenced-cells. Besides, the anti-tumor effects of lncRNA UCA1 silence towards EOMA cells were reversed by miR-200c suppression. Same effects of lncRNA UCA1 and miR-200c on HemECs cells were observed. Furthermore, silence of lncRNA UCA1 repressed mTOR, AMPK and Wnt/β-catenin signaling via a miR-200c-dependent fashion.

Conclusion: This study evidences that silence of lncRNA UCA1 inhibits hemangioma cells growth, migration and invasion possibly via its regulation on miR-200c expression and the activation of mTOR, AMPK and Wnt/β-catenin signaling pathways.

1. Introduction

Infantile hemangioma (IH) is the most common benign tumor of infancy that is caused by hyperproliferation of mesoblastic vascular tissues. The incidence of IH among infants is about 4–5% [1,2], and it is more frequently in females and Caucasians [2,3]. IH may occur in various regions of the body, including head, face, neck, and limbs. Management of IH is highly individualized and may include pharmacotherapy or surgery [4]. But surgery is not entirely curative for symptoms, as it has been reported that 25% of those undergoing resection had persistence of symptoms [5]. A better understanding of IH is warranted for discovery of novel treatment.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs with > 200 nucleotides. In the last decade, lncRNAs have been reported as significant regulators in multiple biological process, such as cell proliferation, cell-cycle progression, apoptosis, differentiation and even the initiation of cancers [6], which furthered our understanding of human cancers. Human urothelial carcinoma associated 1 (UCA1) is an lncRNA mapped to chromosome region of 19p13.12, that is originally found in human bladder cancer [7]. It has been reported that lncRNA UCA1 has an oncogenic function in various cancers, including cholangiocarcinoma [8], oral squamous cell carcinoma [9], gallbladder cancer [10], and gastric cancer [11]. But the role of lncRNA UCA1 in IH has not been revealed yet.

microRNAs (miRNAs) are another kind of non-coding RNAs, with approximately 22 nucleotides in length. It is well-known that miRNAs play critical role in the post-transcriptional regulation of gene expression by binding with the 3'UTR region of mRNAs. More interestingly, lncRNAs can sequester miRNAs away from their target mRNAs by binding and antagonizing miRNAs [12]. In contrast to lncRNA UCA1, miR-200c has been demonstrated as a tumor suppressive gene in many cancer types, that miR-200c suppressed tumor cells metastasis and

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https://doi.org/10.1016/j.lfs.2019.03.038
Received 24 December 2018; Received in revised form 14 March 2019; Accepted 16 March 2019
Available online 18 March 2019
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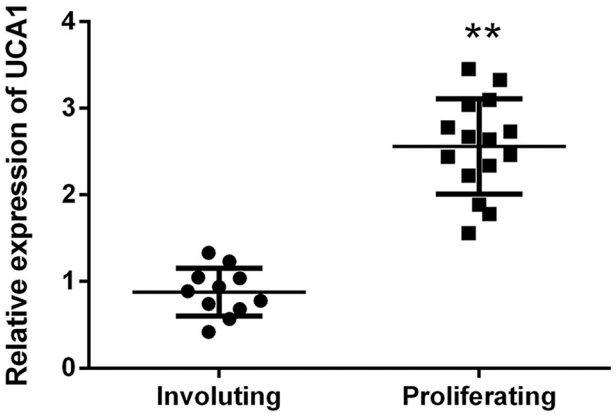

Fig. 1. Expression of lncRNA UCA1 in human IH tissues. The expression levels of lncRNA UCA1 in hemangioma samples collected from 15 infants with proliferating-phase IH and 12 infants with involuting-phase IH were measured by qRT-PCR. ** $p < 0.01$ vs. Involuting group.

growth [13–15]. In addition, miR-200c has gained consideration as a regulator of epithelial-to-mesenchymal transition, which is a biological process responsible for tumor progression, and tumor cells invasion and migration [16].

This study aimed to explore the functional role of lncRNA UCA1 in hemangioendothelioma cells. Besides, we tried to decode the underlying mechanisms of lncRNA UCA1's function by decode the relationship between lncRNA UCA1 and miR-200c.

2. Materials and methods

2.1. Patient sample

Hemangioma samples were surgically collected from 15 patients with proliferating-phase IH (13 females and 2 males; median age, 6 months) and 12 patients with involuting-phase IH (10 females and 2 males; median age, 7 months), respectively. This study was approved by the Ethics Committee of Weifang People's Hospital and all procedures were performed in accordance with the ethical standards recommended. Written informed consent was obtained from each individual's guardians.

2.2. Cell culture

Mouse hemangioendothelioma cell line EOMA (CRL-2586™, ATCC, Manassas, VA) was routinely cultured in DMDM (Gibco, Grand Island, NY) with 10% heat-inactivated fetal bovine serum (FBS, Gibco). Hemangioma-derived endothelial cells (HemECs) were isolated from proliferating-phase hemangioma of 3 infants, as previously described [17]. HemECs cells were cultured in Endothelial Basal Medium-2 (EBM-2; Cambrex Bio Science, Walkersville, MD) supplemented with 10% FBS. Both EOMA and HemECs cells were maintained at 37 °C in a humid CO_2 incubator (HF-90, HealForce, Hong Kong, China). The cells were cultured in 75 cm^2 flask (Corning, New York), and subculture was performed by using Trypsin-EDTA solution (Sigma-Aldrich, St. Louis, MO) every other day.

2.3. Transfection

Two shRNA sequences specific for lncRNA UCA1 (sh-UCA1#1 and sh-UCA1#2) were designed and synthesized by GenePharma Co (Shanghai, China), they were respectively inserted into pGPU6 plasmid (GenePharma). A non-targeting shRNA sequence inserted into pGPU6 was used as a negative control (sh-NC). A plasmid for expression of lncRNA UCA1 was constructed by inserting full-length of lncRNA UCA1 in pcDNA3.1 vector (Life Technologies Corporation, Carlsbad, CA). The empty pcDNA3.1 was used as its negative control. miR-200c inhibitor, miR-200c mimic and the scrambled negative controls (NC and mimic NC) were also purchased from GenePharma. Transfection was performed in 6-well plates, under non-serum and non-antibiotic conditions. Lipofectamine 3000 reagent (Life Technologies Corporation) was used in this process, according to the manufacturer's instructions. The final concentration of plasmid and miRNAs used for transfection was 100 nM and 2 μg/mL, respectively. At 48 h of transfection, the transfected cells were established.

2.4. qRT-PCR

Total RNAs were extracted by Trizol reagent (Life Technologies Corporation, Carlsbad, CA). Reverse transcription and qRT-PCR were performed by using PrimerScript 1st Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, CA) and SYBR ExScript RT-qPCR Kit (Takara, Dalian, China). The internal control used in this system was U6 for miR-200c and β-actin for lncRNA UCA1. Data was calculated according to the $2^{-\Delta\Delta Ct}$ method [18].

2.5. CCK-8 assay

The transfected cells were planted into 96-well plates with a density of 5×10^3 cells/well, then the cells were allowed for culturing at 37 °C for 48 h. The culture medium in each well was removed, the cells were

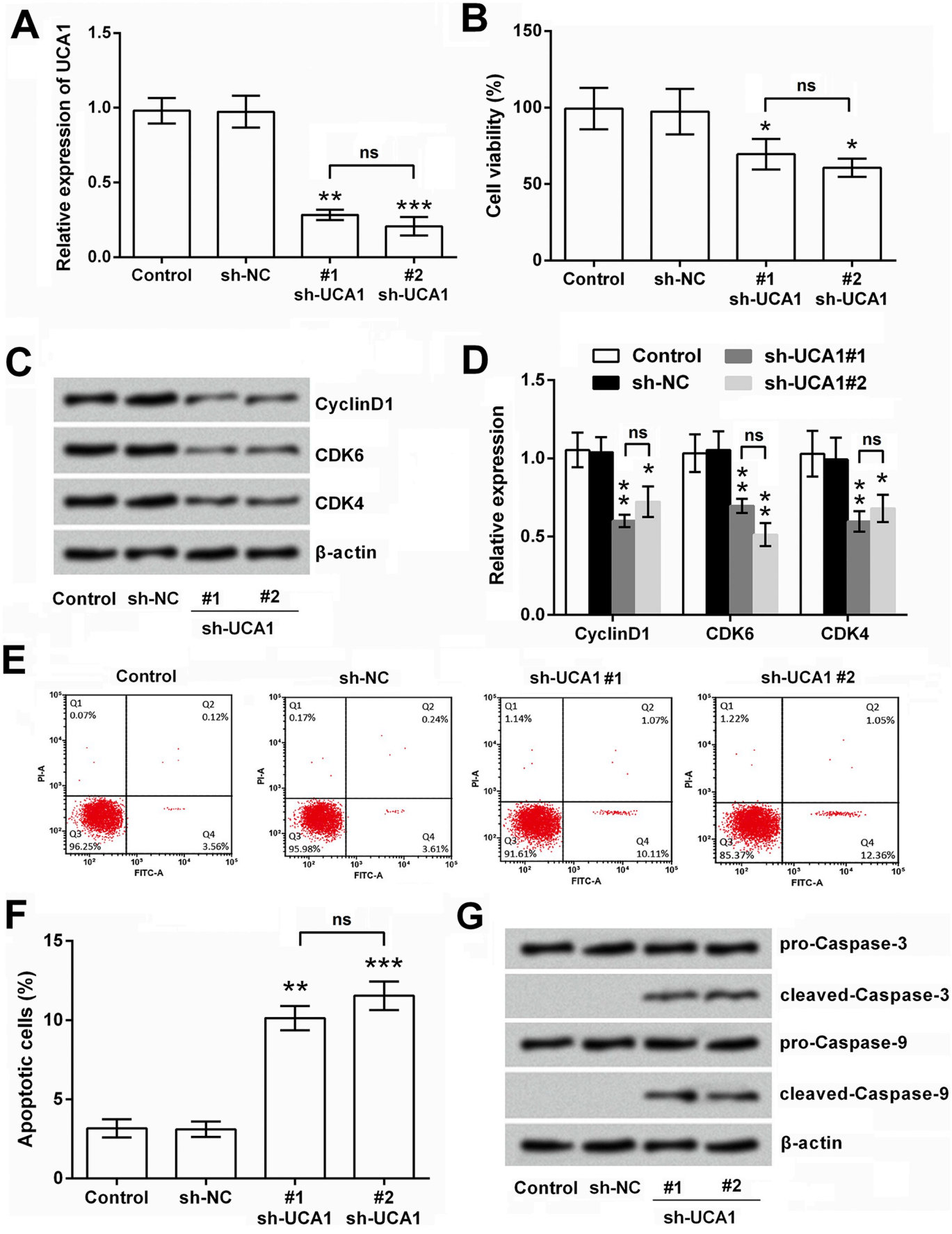

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Fig. 2. Effect of lncRNA UCA1 silence on EOMA cells proliferation and apoptosis. EOMA cells were transfected with two shRNA sequences specific for lncRNA UCA1 (sh-UCA1#1 and sh-UCA1#2) or its negative control (sh-NC). Then, (A) lncRNA UCA1 expression changes were detected by qRT-PCR; (B) cell viability was detected by CCK-8 assay; (C-D) expression levels of cell-cycle-related proteins were assessed by Western blot; (*E*-F) apoptotic cell rate was measured by flow cytometry; and (G) expression levels of apoptosis-related proteins were assessed by Western blot. ns, no significance vs. the indicated group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. sh-NC group.

washed twice by phosphate buffer saline (PBS), and 10 μL CCK-8 solution (Dojindo Molecular Technologies, Kyushu, Japan) was added into each well. The plates were incubated at 37 °C for 4 h, after which the absorbance of each well was measured by Microplate Reader (Bio-Rad, Hercules, CA) at 450 nm.

2.6. Apoptosis assay

After transfection, 1×10^5 cells were collected from each group, and the apoptotic cell rate was measured by using Annexin V-FITC Apoptosis Detection Kit (Beyotime, Shanghai, China). The collected cells were resuspended in 200 μL Binding Buffer containing 10 μL Annexin V-FITC. The samples were incubated at room temperature for 30 min in the dark, and then 300 μL PBS and 5 μL PI was added. The samples were then immediately detected by a FACS can (Beckman Coulter, Fullerton, CA), and the data was read by FlowJo software (Tree Star, San Carlos, California).

2.7. Transwell assay

The migratory and invasive capacity of transfected cells was detected by using a modified Boyden chamber (Costar-Corning, New York), which with 24 wells and 8.0-μm pore polycarbonate flter inserts. For the detection of cell migration, 1×10^3 transfected cells were seeded in the upper side of the chamber and were cultured under non-serum condition. The lower chamber was filled with 600 μL DMEM plus 10% FBS. After 24 h of incubation at 37 °C, the migrated cells in the lower chamber were stained by 1% crystal violet (Beyotime) for 30 min. The stained cells were counted microscopically from five randomly selected fields. Cell invasion was detected the same as cell migration, except that before assessment, the insert was pre-coated with Matrigel (Millipore, MA).

2.8. Western blot

After transfection, cells were collected and the protein in cell was extract by RIPA lysis buffer (Beyotime). The amount of protein was determined by the BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Equal amount of proteins were loaded and separated by SDS-PAGE, and were transferred onto PVDF membrane (Millipore, Billerica, MA). The protein loaded membranes were incubated in 5% non-fat milk for 1 h at room temperature, and then were proved by primary antibodies at 4 °C overnight. Anti-pro-caspase-3 (ab32499), anti-cleaved-caspase-3 (ab13847), anti-pro-caspase-9 (ab2013), anti-cleaved-caspase-9 (ab2324), anti-p-p70S6K (ab2571), anti-p70S6K (ab14708), anti-p-mTOR (ab109268), anti-mTOR (ab32028), anti-p-AMPK (ab92701), anti-AMPK (ab32047), anti-Wnt3a (ab219412), anti-β-catenin (ab16051) and anti-β-actin (ab8227) were obtained from Abcam (Cambridge, MA); anti-MMP-9 (sc-6840), anti-Vimentin (sc-7557), anti-CyclinD1 (sc-717), anti-CDK6 (sc-177), and anti-CDK4 (sc-260) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The membranes were then incubated with the goat anti-rabbit IgG (ab6721, Abcam) and donkey anti-goat IgG (ab6881, Abcam) for 1 h at room temperature. The positive bands were developed with enhanced chemiluminescence detection kit (Thermo scientific, Rockford, IL), and the intensity was quantified using Image Lab™ Software (Bio-Rad, Hercules, CA).

2.9. Statistics

All experiments were repeated three times. Statistics were performed by using SPSS 19.0 software (SPSS Inc., Chicago, IL). Statistical differences between groups were analyzed by ANOVA. A *p*-value of < 0.05 was considered as a statistical result.

3. Results

3.1. LncRNA UCA1 was up-regulated in human IH tissues

The expression changes of lncRNA UCA1 in human IH tissues were tested by qRT-PCR. Data in Fig. 1 showed that, the expression of lncRNA UCA1 was significantly up-regulated in proliferating-phase hemangioma samples, as compared to involuting-phase hemangioma tissues ($p < 0.01$). These in vivo data suggested the importance of lncRNA UCA1 in the progression of IH.

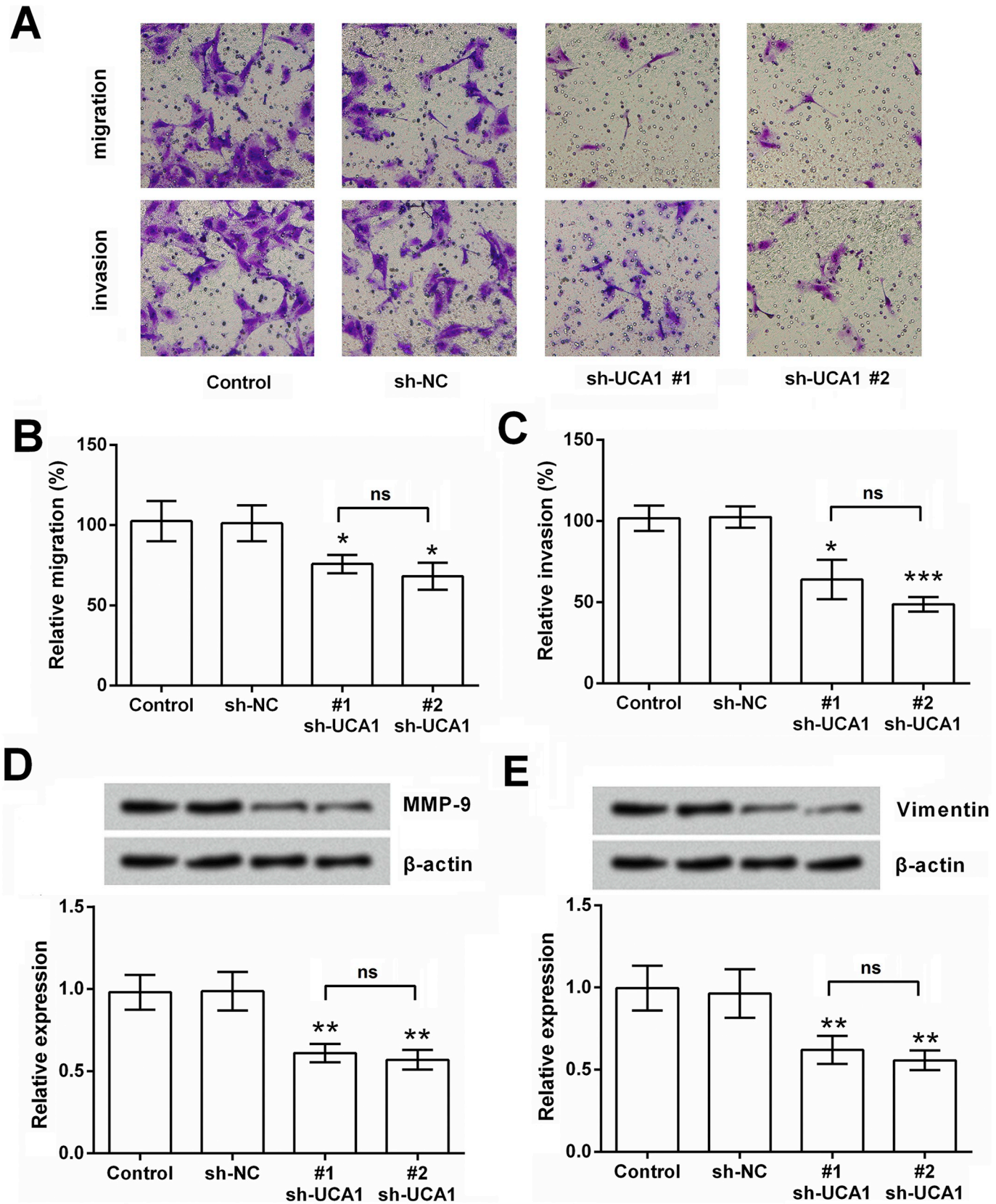

Fig. 3. Effect of lncRNA UCA1 silence on EOMA cells migration and invasion. EOMA cells were transfected with sh-UCA1#1, sh-UCA1#2 or sh-NC. Then, (A-C) relative migration and invasion were determined by Transwell assay; expression levels of (D) MMP-9 and (E) Vimentin proteins were assessed by Western blot. ns, no significance vs. the indicated group.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. sh-NC group.

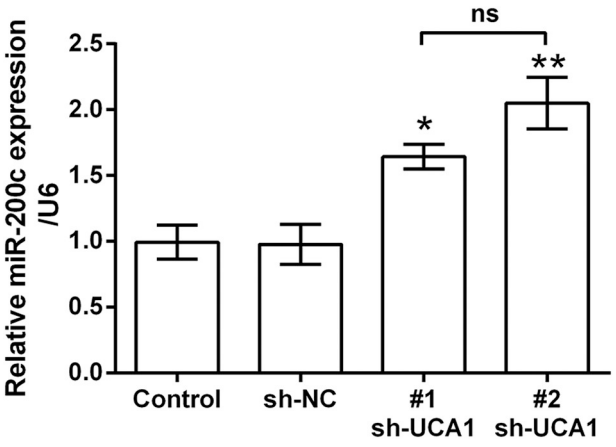

Fig. 4. Effect of lncRNA UCA1 silence on miR-200c expression. EOMA cells were transfected with sh-UCA1#1, sh-UCA1#2 or sh-NC, after which the expression levels of miR-200c were determined by qRT-PCR. ns, no significance vs. the indicated group.* $p < 0.05$, ** $p < 0.01$ vs. sh-NC group.

3.2. Silence of lncRNA UCA1 inhibited EOMA cells proliferation and promoted apoptosis

Two shRNA sequences specific for lncRNA UCA1, i.e., sh-UCA1#1 and sh-UCA1#2 were respectively transfected into EOMA cells. Fig. 2A showed that, the lncRNA UCA1 expression levels were much lower in sh-UCA1#1 and sh-UCA1#2 groups than in the sh-NC group ($p < 0.01$ and $p < 0.001$), indicating lncRNA UCA1 was successfully silenced by shRNA transfection. By performing CCK-8 assay, we found that the viability of EOMA cells was significantly reduced by sh-UCA1#1 and sh-UCA1#2, when compared to the sh-NC group (both $p < 0.05$, Fig. 2B). Then, the expression levels of cell-cycle-related proteins were detected by Western blot, to reveal whether silence of lncRNA UCA1 could alter cell-cycle progression. As shown in Fig. 2C-D, the protein levels of CyclinD1, CDK6 and CDK4 were all significantly reduced by sh-UCA1#1 and sh-UCA1#2, when compared to sh-NC ($p < 0.05$ or $p < 0.01$). Next, flow cytometry was conducted to detect cell apoptosis post-transfection. We found that the apoptosis rates were significantly increased after transfection with sh-UCA1#1 and sh-UCA1#2, when compared to the sh-NC transfection ($p < 0.01$ and $p < 0.001$, Fig. 2E-F). Also, caspase-3 and caspase-9 were remarkably cleaved in EOMA cells after transfection with sh-UCA1#1 and sh-UCA1#2 (Fig. 1G). These data collectively suggested that silence of lncRNA UCA1 induced a significant cell lose by inhibiting proliferation and inducing apoptosis.

3.3. Silence of lncRNA UCA1 inhibited EOMA cells migration and invasion

Transwell assay was performed to assess the migratory and invasive capacities of EOMA cells post-transfection. As seen in Fig. 3A-3C, the relative migration and invasion were both reduced significantly by sh-UCA1#1 and sh-UCA1#2, when compared to the sh-NC ($p < 0.05$ or $p < 0.001$). Fig. 3D-3E showed that, the protein expression levels of MMP-9 and Vimentin were both significantly down-regulated by sh-UCA1#1 and sh-UCA1#2 (all $p < 0.01$).

3.4. Silence of lncRNA UCA1 up-regulated the expression of miR-200c

The expression changes of miR-200c in EOMA cells following transfection with sh-UCA1#1 or sh-UCA1#2 were detected by qRT-PCR. Data in Fig. 4 showed that the expression levels of miR-200c were significantly increased by sh-UCA1#1 and sh-UCA1#2, when compared to the sh-NC ($p < 0.05$ and $p < 0.01$). These data give us a clue that the elevated miR-200c expression induced by lncRNA UCA1 silence might be involved in lncRNA UCA1's functions.

3.5. Silence of lncRNA UCA1 inhibited EOMA cells proliferation and promoted apoptosis via up-regulation of miR-200c

In order to verify the above mentioned hypothesis, the expression of miR-200c in cell was suppressed by inhibitor transfection. As qRT-PCR analytical data shown in Fig. 5A, miR-200c expression was successfully reduced by miR-200c inhibitor transfection. Of note, sh-UCA1#1 and sh-UCA1#2 induced cell viability impairment ($p < 0.05$, Fig. 5B), and cell-cycle-related protein up-regulations ($p < 0.05$, $p < 0.01$, or $p < 0.001$, Fig. 5C-D) were all reversed by miR-200c inhibitor transfection. Also, the inducement of apoptosis ($p < 0.05$, Fig. 5E-F) and the cleavage of caspase-3 and caspase-9 (Fig. 5G) induced by sh-UCA1#1 and sh-UCA1#2 were attenuated by miR-200c inhibitor transfection. All these data indicated that the inhibitory effects of lncRNA UCA1 silence on EOMA cells growth were attenuated when miR-200c was silenced.

3.6. Silence of lncRNA UCA1 inhibited EOMA cells migration and invasion via up-regulation of miR-200c

The involvement of miR-200c in the impacts of lncRNA UCA1 on EOMA cells migration and invasion was investigated. As expected, the migration and invasion impairment induced by sh-UCA1#1 and sh-UCA1#2 was alleviated by miR-200c inhibitor ($p < 0.05$, Fig. 6A-C). Besides, sh-UCA1#1 and sh-UCA1#2 induced the down-regulations of MMP-9 and Vimentin were reversed by miR-200c inhibitor ($p < 0.05$ or $p < 0.01$, Fig. 6D-E). These data suggested that the inhibitory effects

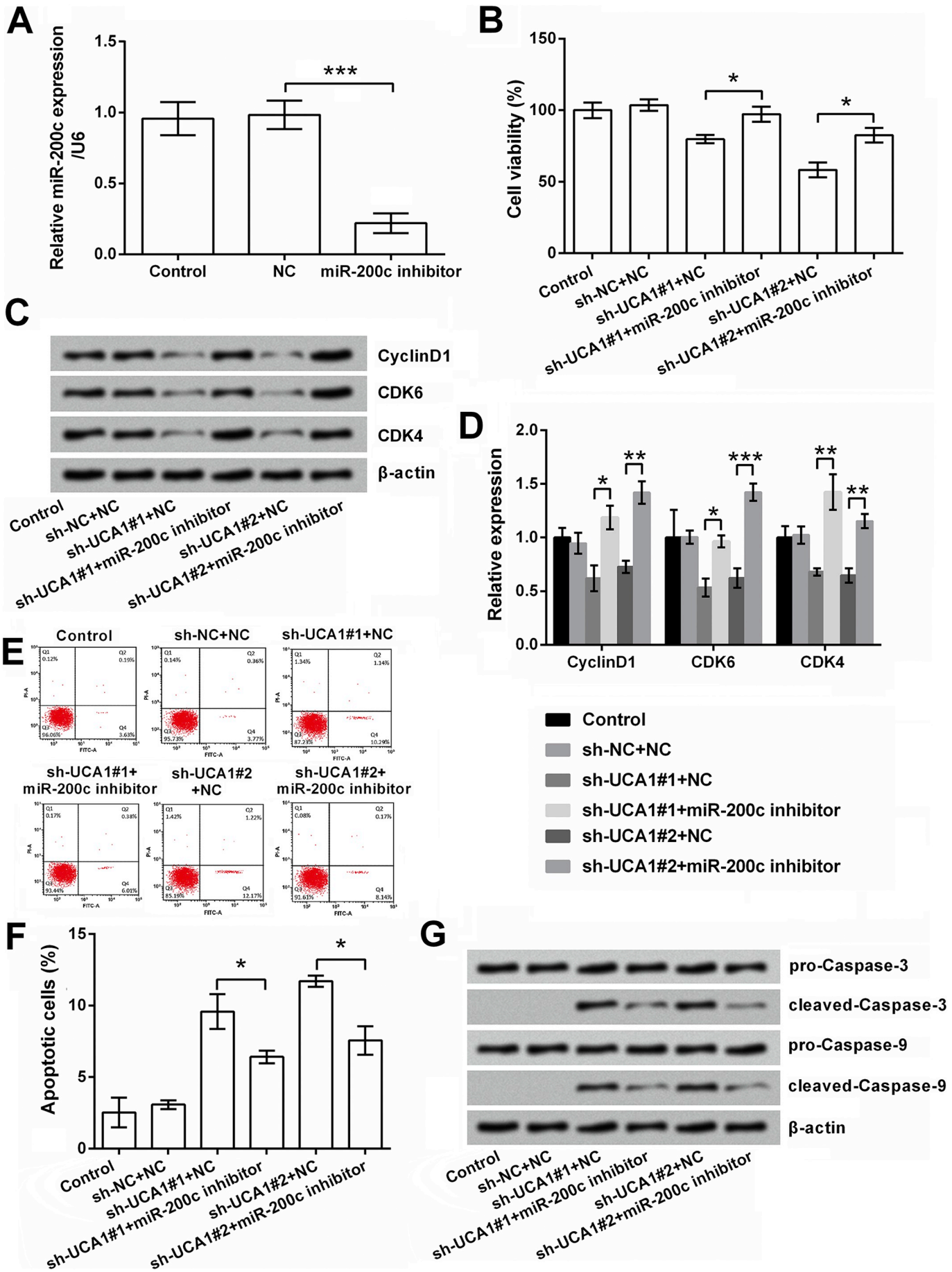

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Fig. 5. Effect of lncRNA UCA1 silence and miR-200c suppression on EOMA cells proliferation and apoptosis. (A) EOMA cells were transfected with miR-200c inhibitor or its negative control (NC), after which miR-200c expression was detected by qRT-PCR. Then, EOMA cells were co-transfected with sh-UCA1#1/2 and miR-200c inhibitor. Post-transfection, (B) cell viability was detected by CCK-8 assay; (C-D) expression levels of cell-cycle-related proteins were assessed by Western blot; (*E*-F) apoptotic cell rate was measured by flow cytometry; and (G) expression levels of apoptosis-related proteins were assessed by Western blot. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the indicated group.

of lncRNA UCA1 silence on EOMA cells migration and invasion were also attenuated when miR-200c was silenced.

3.7. Gain-function of lncRNA UCA1 promoted HemECs cells growth, migration and invasion via down-regulation of miR-200c

Further experiments are performed to see whether the impacts of lncRNA UCA1 on EOMA cells can be reproduced in another hemangioma cell line HemECs. The expression of lncRNA UCA1 and miR-200c was overexpressed by transfection with pcDNA-UCA1 and miR-200c mimic. As data shown in Fig. 7A-7B, up-regulated lncRNA UCA1 and miR-200c was observed following transfection ($p < 0.001$). More importantly, gain-function of lncRNA UCA1 significantly promoted cell proliferation (Fig. 7C-E), while had no significant impacts on cell apoptosis (Fig. 7G-H). However, transfection of cells with miR-200c mimic suppressed cell proliferation and evoked caspases-dependent apoptosis (Fig. 7C-H). Also, lncRNA UCA1 gain-function significantly promoted HemECs cells migration and migration, while the promoting effects were flattened by miR-200c mimic (Fig. 8A-E).

3.8. Silence of lncRNA UCA1 repressed mTOR, AMPK and Wnt/β-catenin signaling via up-regulation of miR-200c

For further exploration, we focused on the impact of lncRNA UCA1 silence on the activation of mTOR, AMPK and Wnt/β-catenin signaling pathways. Western blot results showed that, the phosphorylation levels of p70S6K, mTOR and AMPK as well as the expression levels of Wnt3a and β-catenin were all reduced by sh-UCA1#1 and sh-UCA1#2 transfection ($p < 0.05$ or $p < 0.01$, Fig. 9A-9C). Additionally, the reduced levels by sh-UCA1#1 and sh-UCA1#2 were reversed by miR-200c inhibitor ($p < 0.01$ or $p < 0.001$).

4. Discussion

Recent years, a great deal of evidence has proposed that lncRNA UCA1 is an oncogenic gene [8–11], and miR-200c is a tumor suppressive gene [13–16]. However, the role of lncRNA UCA1 and miR-200c in IH remains largely unknown. Thus, this study aimed to explore the functional effects of lncRNA UCA1 and miR-200c on hemangioendothelioma cells growth, migration and invasion. We found that lncRNA UCA1 was up-regulated in proliferating-phase hemangioma samples, as compared to involuting-phase. Silence of lncRNA UCA1 significantly reduced EOMA cells proliferation, migration and invasion, and significantly induced apoptosis. miR-200c was up-regulated in response to lncRNA UCA1 silence. The tumor suppressive effects of lncRNA UCA1 silence towards EOMA cells were reversed by miR-200c suppression. The anti-tumor effects of lncRNA UCA1 were also reproduced in HemECs cells by performing gain-function experiment. Moreover, silence of lncRNA UCA1 repressed mTOR, AMPK and Wnt/βncatenin signaling via a miR-200c-dependent fashion.

During the life cycle of IH, proliferation and involution are two main phases [19]. Indeed, CyclinD1, CDK4, and CDK6 have been reported to control the vascular endothelial cells proliferation during pathogenic neovascularization [20]. The current findings showed that lncRNA UCA1 was highly expressed in proliferating-phase hemangioma samples than involuting-phase, suggesting lncRNA UCA1 might be participated in the progression of IH. The further in vitro investigation performed in EOMA and HemECs cells indicated that silence of lncRNA UCA1 was capable of reducing hemangioendothelioma cells proliferation via modulation cell-cycle-related protein expressions, including CyclinD1, CDK4, and CDK6. Apoptosis has been recommended and privileged as another strategy for clearing tumor cells [21]. Apoptosis of human hemangioma endothelial cells increased significantly in the involution phase than that in the proliferation phase [1]. Previous studies have demonstrated that lncRNA UCA1 facilitated apoptosis via

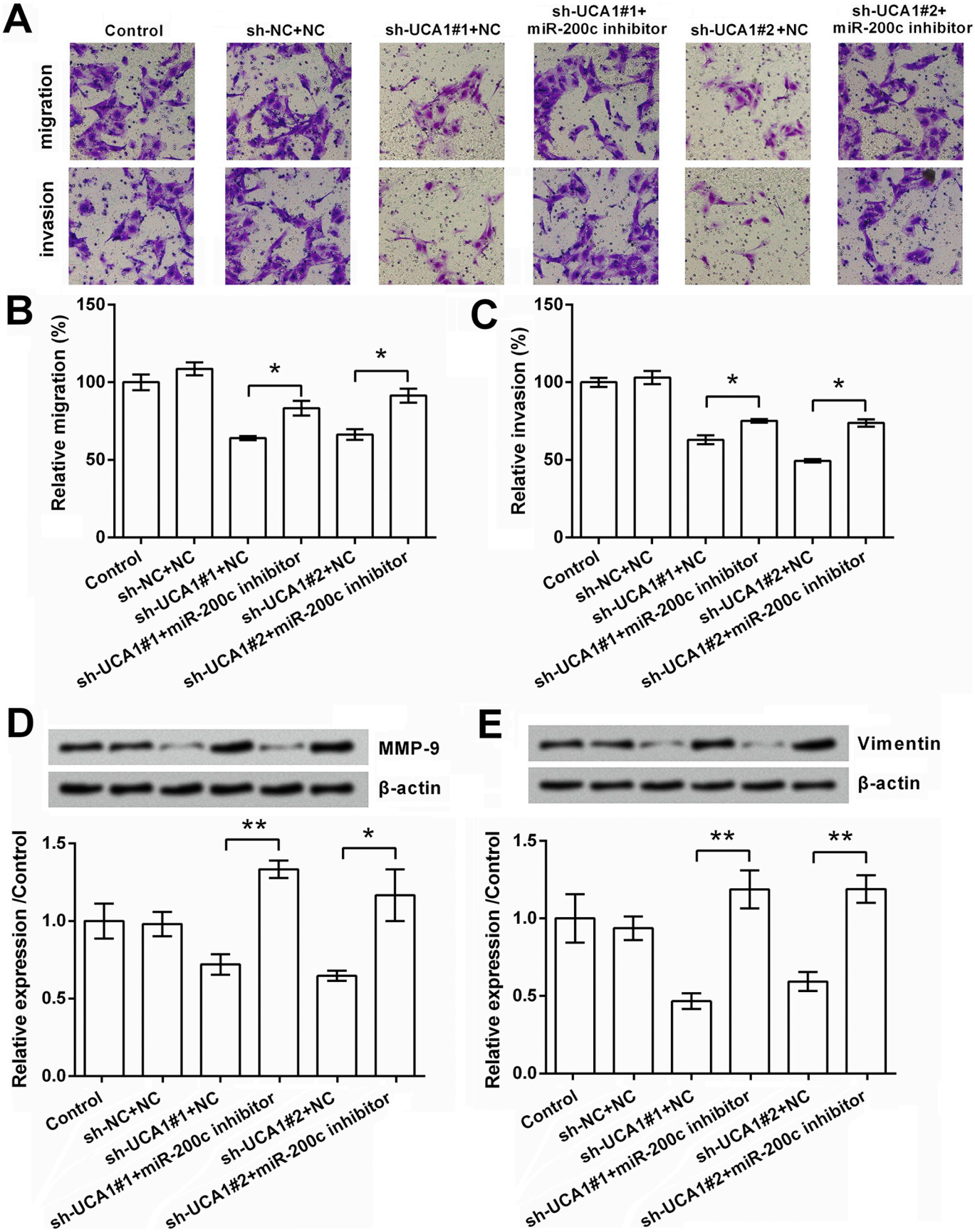

Fig. 6. Effect of lncRNA UCA1 silence and miR-200c suppression on EOMA cells migration and invasion. EOMA cells were co-transfected with sh-UCA1#1/2 and miR-200c inhibitor. Post-transfection, (A-C) relative migration and invasion were determined by Transwell assay; expression levels of (D) MMP-9 and (E) Vimentin proteins were assessed by Western blot. * $p < 0.05$, ** $p < 0.01$ vs. the indicated group.

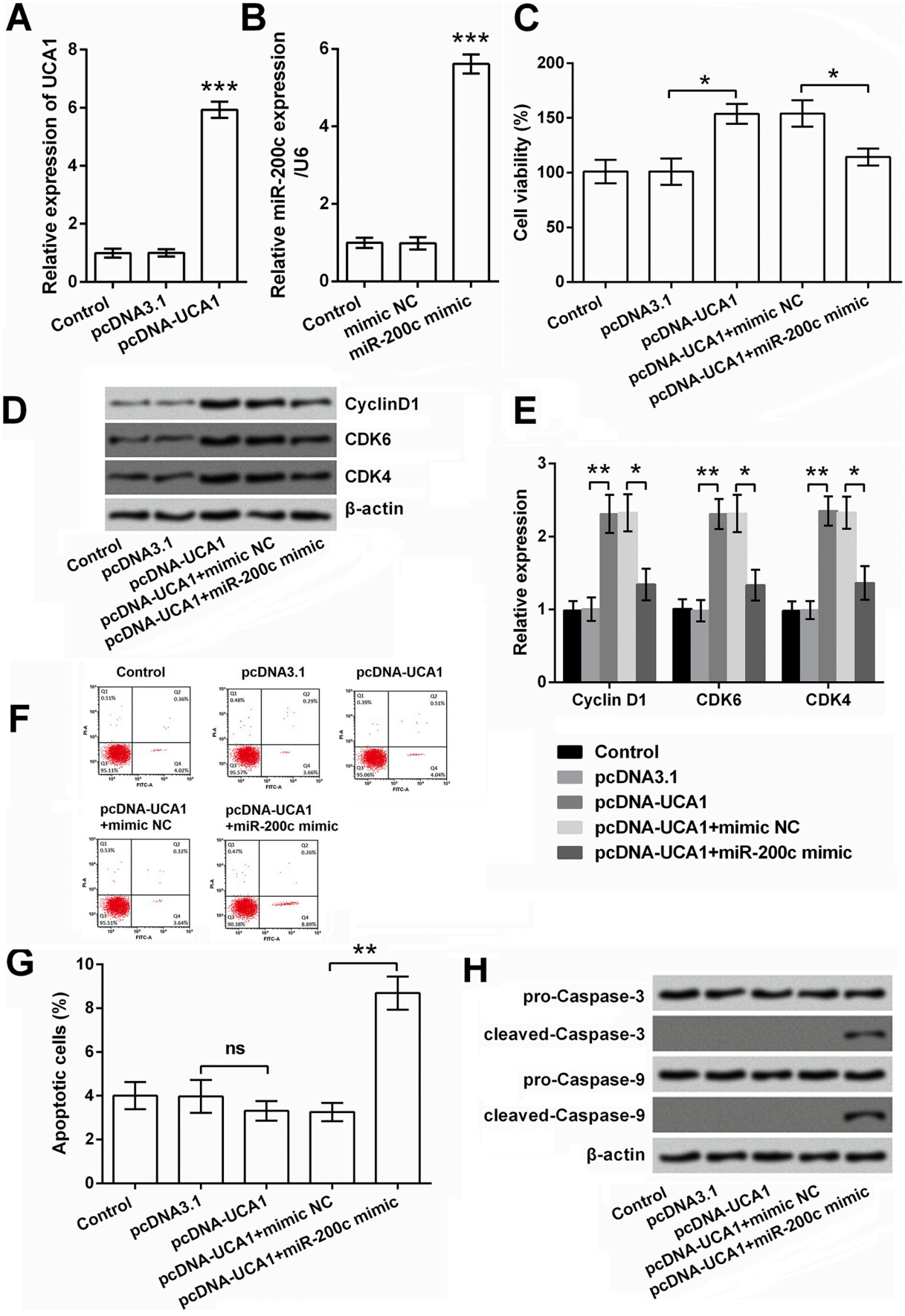

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Fig. 7. Effect of lncRNA UCA1 gain-function and miR-200c overexpression on HemECs cells proliferation and apoptosis. (A) HemECs cells were transfected with a plasmid for expression of lncRNA UCA1 (pcDNA-UCA1) or the empty pcDNA3.1 vector, after which lncRNA UCA1 expression was detected by qRT-PCR. (B) HemECs cells were transfected with miR-200c mimic or its negative control (mimic NC), after which miR-200c expression was detected by qRT-PCR. Then, HemECs cells were co-transfected with pcDNA-UCA1 and miR-200c mimic. Post-transfection, (C) cell viability was detected by CCK-8 assay; (D-E) expression levels of cell-cycle-related proteins were assessed by Western blot; (F-G) apoptotic cell rate was measured by flow cytometry; and (H) expression levels of apoptosis-related proteins were assessed by Western blot. ns, no significance; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the indicated group.

enhancing caspase-3 cleavage in hepatocellular carcinoma cells [22] and cholangiocarcinoma cells [8], as well as via inducing expression of cleaved caspase-9 in breast cancer cells [23]. This phenomenon was also observed in this study, as silence of lncRNA UCA1 significantly increased apoptosis rate and remarkably cleaved caspase-3 and caspase-9. In addition to the functional role in tumor cells proliferation and apoptosis, lncRNA UCA1 also implicates in tumor cells migration and invasion [24,25]. In consistence with these previous studies, our findings for the first time evidenced that silence of lncRNA UCA1 inhibited hemangioendothelioma cells migration and invasion. Besides, such inhibition may be due to the decreased protein expression of MMP-9 and Vimentin.

Increasing studies showed that lncRNA UCA1 functioned as an oncogenic gene in various cancers by modulation of miRNAs expression. Gu et al., indicated that elevated expression of lncRNA UCA1 promoted gastric cancer cells proliferation, colony formation ability, and invasion via sponging miR-590-3p [26]. Li et al., demonstrated that lncRNA UCA1 enhanced mitochondrial function and cell viability in bladder cancer cells via inhibition of miR-195 signaling [27]. Herein, miR-200c, a widely reported tumor suppressive miRNA [13–15], was identified as another downstream gene for lncRNA UCA1. miR-200c expression was down-regulated by lncRNA UCA1 silence, and the anti-tumor actions induced by lncRNA UCA1 silence in hemangioendothelioma cells were reversed by miR-200c suppression.

mTOR is a member of PI3K-related kinase (PIKK) family, and is activated by stress, energy, amino acids, DNA damage, or growth factors [28]. It has been reported that dysregulation of mTOR is linked to tumorgenesis [29]. Medici and Olsen have mentioned that rapamycin (an inhibitor of mTOR) reduced hemangioma endothelial cells proliferation and VEGF levels [30]. Furthermore, the oncogenic functions of lncRNA UCA1 in breast cancer cells [23], gastric cancer cells [31], and small cell lung cancer cells [32] have been reported to be realized through positively regulation of mTOR pathway. AMPK is a crucial cellular energy sensor that regulates the balance of metabolic energy [33]. The activation of AMPK is implicated in many health benefits, made AMPK as a promising target for treating diabetes and cancer [34,35]. Wnt/β-catenin is another signaling pathway significant involved in the pathogenesis of human cancers [36,37]. When Wnt signaling is activated, accumulated β-catenin translocate from cytoplasm to nucleus, and make complex with TCF/LEF and then active the transcription of target genes. The current findings indicated that silence of lncRNA UCA1 deactivated mTOR, AMPK and Wnt/β-catenin signaling pathways, while the deactivation of these signaling was reversed by miR-200c inhibition. These findings suggested that silence of lncRNA UCA1 reduced the activation of mTOR, AMPK and Wnt/β-catenin signaling pathways in a miR-200c-dependent fashion.

In conclusion, this study provides in vitro evidence that silence of lncRNA UCA1 inhibits hemangioendothelioma cells growth, migration and invasion. The possible mechanism of the action may be via its regulation on miR-200c expression and the activation of mTOR, AMPK and Wnt/β-catenin signaling pathways. This study provides us a better understanding of the role of lncRNA UCA1 in cancer treatment.

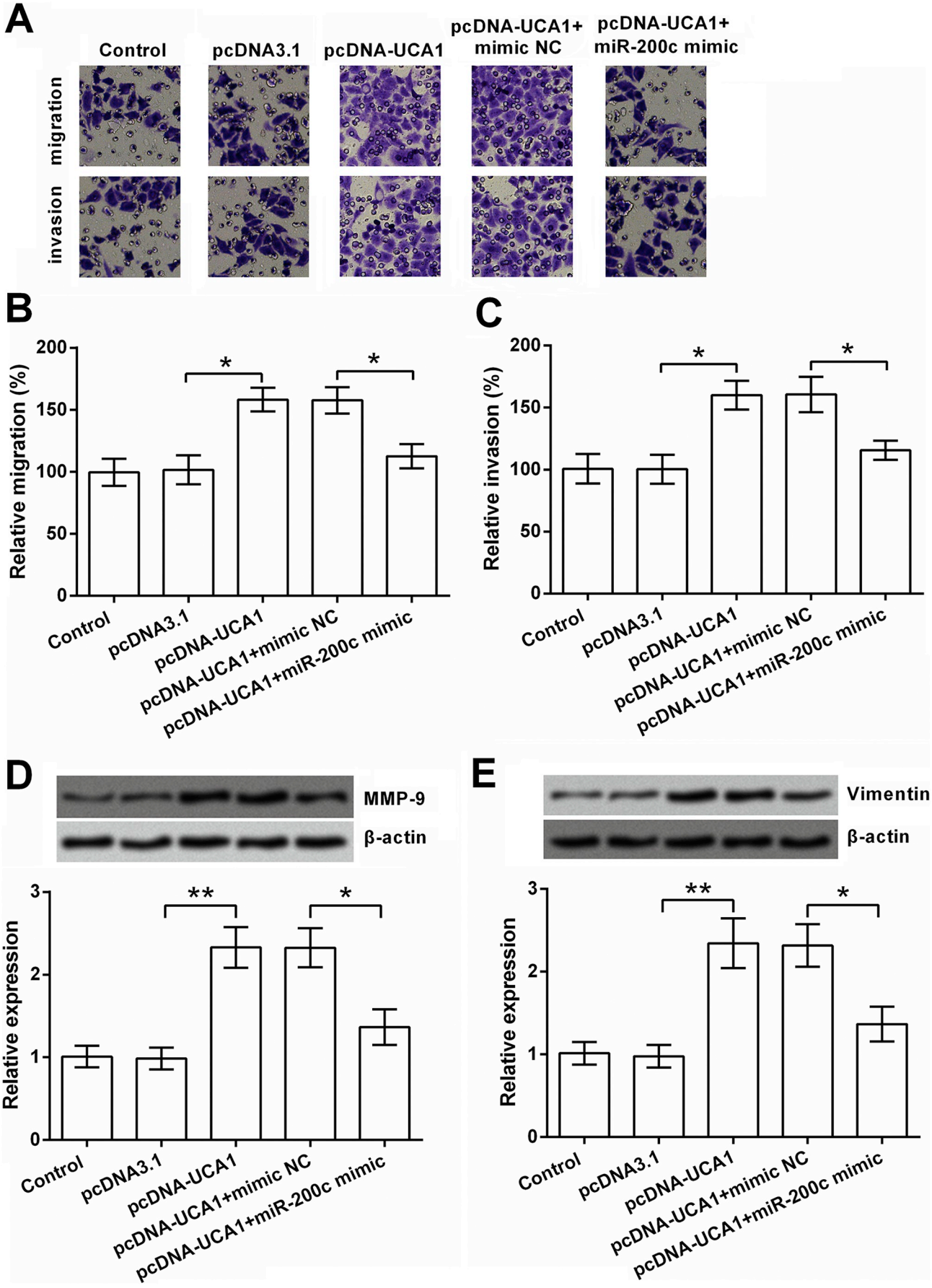

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Fig. 8. Effect of lncRNA UCA1 gain-function and miR-200c overexpression on HemECs cells migration and invasion. HemECs cells were co-transfected with pcDNA-UCA1 and miR-200c mimic. Post-transfection, (A-C) relative migration and invasion were determined by Transwell assay; expression levels of (D) MMP-9 and (E) Vimentin proteins were assessed by Western blot. * $p < 0.05$, ** $p < 0.01$ vs. the indicated group.

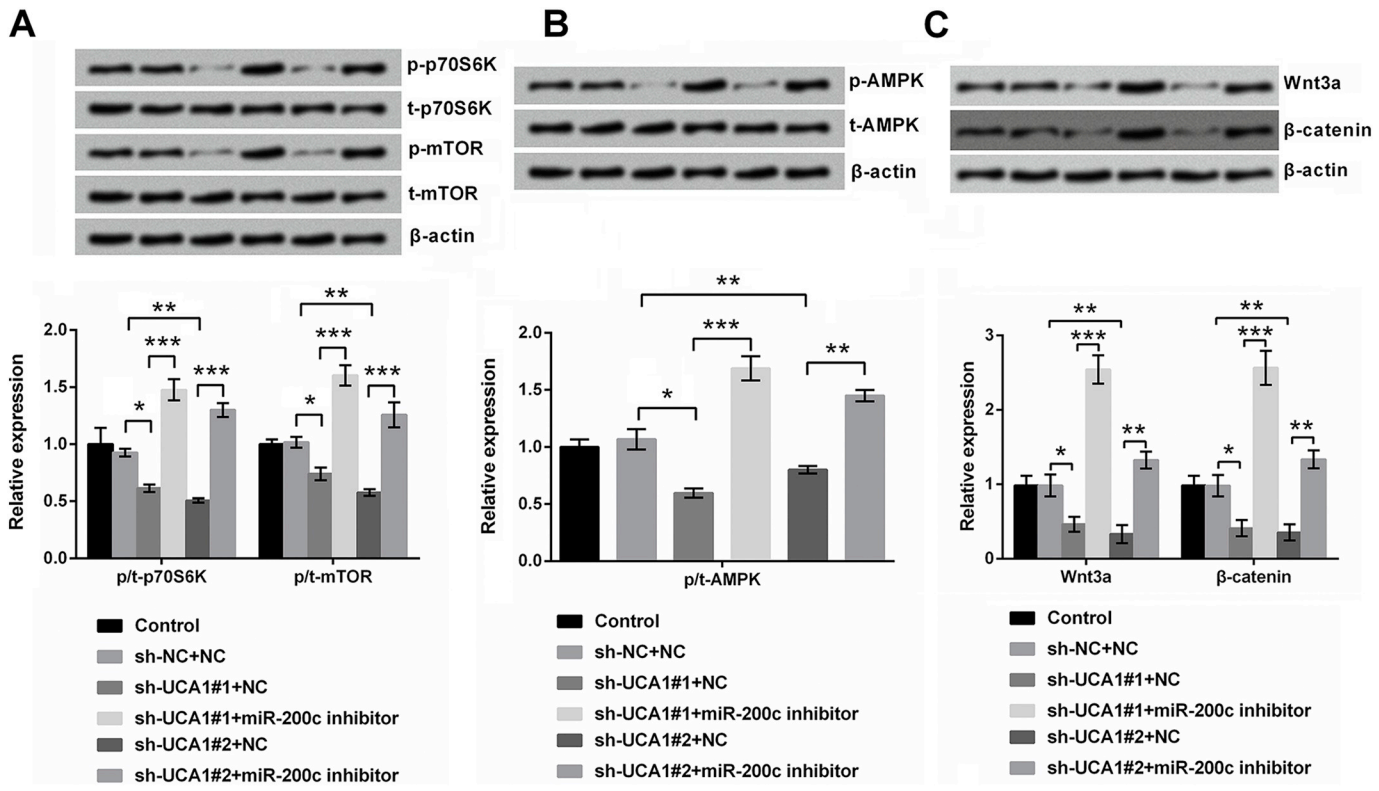

Fig. 9. Effect of lncRNA UCA1 silence and miR-200c suppression on the activation of mTOR, AMPK and Wnt/β-catenin signaling. EOMA cells were co-transfected with sh-UCA1#1/2 and miR-200c inhibitor. Post-transfection, (A) the phosphorylation levels of p70S6K, mTOR and (B) AMPK, (C) as well as the expression levels of Wnt3a and β-catenin were assessed by Western blot. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. the indicated group.

Acknowledge

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

All authors declare that they have no conflict of interest.

Author contributions

Conceived and designed the experiments: Jing Zhang and Chuanguang Zhang; Performed the experiments and analyzed the data: Jing Zhang; Contributed reagents/materials/analysis tools: Chuanguang Zhang; Wrote the manuscript: Jing Zhang; Revised the manuscript: Chuanguang Zhang.

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