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Long Non-Coding RNA Metastasis Associated Lung Adenocarcinoma Transcript 1 (MALAT1) Promotes Hypertension by Modulating the Hsa-miR-124-3p/Nuclear Receptor Subfamily 3, Group C, Member 2 (NR3C2) and Hsa-miR-135a-5p/NR3C2 Axis

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: This study was designed to investigate the role of long non-coding RNA (lncRNA) metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) in the proliferation as well as apoptosis of human umbilical vein endothelial cells (HUVECs), to offer a basis for therapy of hypertension.

Material/Methods: The lncRNA *MALAT1* expression, hsa-miR-124-3p, hsa-miR-135a-5p, hsa-miR-135b-5p, and hsa-miR-455-5p in plasma were measured from 230 patients with hypertension and 230 non-hypertensive controls. The mechanism for lncRNA *MALAT1* modulating the proliferation and apoptosis of HUVECs was explored by cell transfection, Cell Counting Kit-8 (CCK-8), quantitative real-time polymerase chain reaction (qRT-PCR), western blot, and dual-luciferase reporter assays.

Results: The expression of hsa-miR-124-3p and hsa-miR-135a-5p was reduced and the expression of lncRNA *MALAT1* was increased in the plasma of hypertensive patients. Moreover, the plasma levels of hsa-miR-124-3p and hsa-miR-135a-5p of hypertensive patients were negatively correlated with lncRNA *MALAT1* (r=–0.64, –0.72; $P<0.01$, $P<0.01$, respectively). The level of nuclear receptor subfamily 3, group C, member 2 (NR3C2) protein was negatively correlated with hsa-miR-124-3p and hsa-miR-135a-5p (r=–0.74, –0.84; $P<0.01$, $P<0.01$, respectively). The proliferation of HUVECs was inhibited after the inhibition of MALAT. Additionally, after knocking down MALAT, the levels of hsa-miR-124-3p and hsa-miR-135a-5p in HUVECs were markedly increased, while the expression level of NR3C2 protein was decreased. The apoptotic rate of HUVECs after the transfection of MALAT1 small interfering RNA (si-MALAT1) (3.64±0.21%) was significantly reduced compared to that of transfected si-MALAT1 no template control (NC) (3.76±0.19%) and the control group (10.51±1.24%).

Conclusions: LncRNA *MALAT1* regulates proliferation and apoptosis of HUVECs through the hsa-miR-124-3p/NR3C2 and/or hsa-miR-135a-5p/NR3C2 axis.

MeSH Keywords: MicroRNAs • RNA, Long Noncoding • White Coat Hypertension

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Background

Essential hypertension is a chronic disease and a key risk factors for cardiovascular and cerebrovascular diseases. The common complications of hypertension are stroke, chronic kidney disease, heart failure, and myocardial infarction. The disability and mortality rates of hypertension are relatively high, and this disease severely strains medical and social resources, causing a burden on the patients' families and on society [1–3]. Recently, with the aging of the population in China, and improvement in living standards as well as an accelerated pace of life, the incidence of essential hypertension has increased. Moreover, the proportion of younger patients has increased significantly. Researchers are now strongly focusing on the prevention and treatment of essential hypertension.

The pathophysiology of the development of hypertension includes vascular endothelial cell injury, smooth muscle cell proliferation, and vascular remodeling [4,5]. Previous studies found that vascular endothelial cell injury contributes to the occurrence and progression of hypertension; and hypertension-induced vascular endothelial injury further promotes the development of hypertension [6]. It has been reported that hypertension-induced dysfunction of vascular endothelial cells disrupts the secretion of nitric oxide and endothelin, which induces vasoconstriction, thereby increasing circulation resistance and promoting the development of hypertension [7,8]. Additionally, in hypertension, the apoptotic rate of microvascular endothelial cells is significantly increased, resulting in a decrease in parallel blood pathways, an increase in peripheral circulation resistance, and an increase in blood pressure [9–11]. Although accumulating evidence shows that hypertension-induced vascular endothelial cell injury participates in the progression of hypertension, its underlying molecular mechanism has not yet been elucidated and requires further studies.

Long non-coding RNAs (lncRNAs) belong to a family of non-coding transcripts (above 200 nucleotides in length), which play an important role in gene expression at various levels, including pre-transcriptional, transcriptional, and post-transcriptional processes [12,13]. Reportedly, lncRNAs are involved in the cardiovascular system. For example, the expression of lncRNAs in endothelial cells can regulate blood vessel growth and functions [14]. During the progression of hypertension, abnormal vascular tone is usually found, and vascular tone is regulated by vascular smooth muscle-mediated vasoconstriction and endothelial cells. We speculate that lncRNAs may regulate vascular tone by affecting endothelial and vascular smooth muscle cellular functions.

Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) is an important lncRNA. It is 6.7 kb in length and is ubiquitously expressed in human and murine cells [15]. It is also involved in tumor proliferation and apoptosis [16]. However, it is unclear whether the proliferation and apoptosis of human umbilical vein endothelial cells (HUVECs) are impacted by MALAT1. According to a previous study using HUVECs, lncRNA *MALAT1* increased oxidized low-density lipoprotein (ox-LDL) induced autophagy via the phosphatidylinositol 3-kinase/(protein kinase B) (PI3K/AKT) signaling pathway [17].

Hsa-miR-124-3p is a brain-specific microRNAs (miRNAs) [18]; it has been suggested that hsa-miR-124-3p may play an important role in neuronal differentiation. The functions of nuclear receptor subfamily 3, group C, member 2 (NR3C2) in the brain are very important. Previous studies have found that hsa-miR-124-3p can bind to the 3 'untranslated region (UTR) of NR3C1 to regulate the expression of NR3C1 [19]. In addition, another study [20] found that hsa-miR-124-3p and hsa-miR-135a-5p were potential regulators of NR3C2 expression. We found that the binding sites of hsa-miR-124-3p and hsa-miR-135a-5p also existed on NR3C2 3'UTR by bioinformatics screening. LncRNA, miRNA, and other non-coding RNAs (ncRNAs) play an important role in hypertension, and ncRNAs have become widely recognized as diagnostic markers for hypertension [21]. Therefore, in this study we aimed to elucidate whether lncRNA *MALAT1* regulates the proliferation and apoptosis of HUVECs through the hsa-miR-124-3p and hsa-miR-135a-5p/NR3C2 axis, which could provide a theoretical target for the treatment of hypertension.

Material and Methods

Study participants

A total of 230 patients with essential hypertension who were admitted to the Hangzhou Hospital of Traditional Chinese Medicine from June 2016 and February 2019 were randomly selected for this study (153 males and 77 females, age range was 40 to 85 years, mean age: 58.03±8.65 years). The diagnostic criteria for essential hypertension were as follows: sitting systolic blood pressure (SBP) ≥140 mmHg and/or diastolic blood pressure (DBP) ≥90 mmHg [22]. Patients were excluded if they had secondary hypertension, coronary heart disease, valvular heart disease, diabetes, cancer, or immune system diseases. A total of 230 non-hypertensive study participants were recruited as the control group, with age and gender matched to hypertensive patients. The control group age ranged from 39 to 82 years (mean age: 57.56±8.06 years). The SBP of the control group was <140 mmHg, and the DBP was <90 mmHg. The exclusion criteria for the control group were as follows: diabetes, heart, kidney, and other organ damage, coronary heart disease, cancer, and immune system disorders. This study was approved by the Medical Ethics Committee of the

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Hangzhou Hospital of Traditional Chinese Medicine. All participants signed the informed consent form.

Cell culture

HUVECs and human embryonic kidney 293 cells (HEK293) were bought from the American Type Culture Collection and cultured with RPMI-1640 medium (Gibco-BRL, USA) containing 10% fetal bovine serum, and cultured in an incubator with 5% CO_2 at 37°C.

Cell transfection

MALAT1 interfering RNA (si-MALAT1) (5'-CAC AGG GAA AGC GAG TGG TTG GTA A-3' and 5'-TTA CCA ACC ACT CGC TTT CCC TGT G-3'), and si-MALAT1 negative control (NC) (5'-GGC CUA AAG UAG CUA UTT-3' and 5'-AUA GCU ACU UUA GGC CTT-3') were purchased from Shanghai GenePharma Company (Shanghai, China) [23]. To study the effect of lncRNA *MALAT1* on cell proliferation in HUVECs, we diluted si-MALAT1 or si-MALAT1 NC to a working concentration of 20 nM. The plasmid containing si-MALAT1 or si-MALAT1 NC was transfected by Lipofectamine 2000 (Invitrogen, USA), a blank template was transfected as a control (Blank). 48 hours after transfection, the cell cultures were harvested after culture in an incubator at 37°C with 5% CO_2.

Detecting the proliferation of HUVECs using Cell Counting Kit-8 (CCK-8) assay

After the transfected HUVECs were cultured for the designated period of time, the cells were digested, isolated, and counted. The cell density was adjusted to 1 x 10^5/mL and then seeded into a 96-well plate at 100 µL per well (around 1×10^4 cells), cultured in an incubator with 5% CO_2 at 37°C. The cells were collected at 0, 24, and 72 hours, and cultured for 4 hours. Cell viability was detected by Cell Counting Kit-8 (CCK-8) assay and analyzed with a Multiscan MK3 microplate reader and the absorbance at 490 nm (A490) was detected.

Luciferase reporter gene assay

Amplification of cDNA fragments of *MALAT1* wild type (WT) and mutant (MUT) containing binding sites of hsa-miR-124-3p and hsa-miR-135a-5p, as well as WT and MUT of NR3C2 3'UTR were cloned into a pmirGLO vector (Promega, USA). HEK293 cells were seeded into a 48-well plate, 24-hours prior to the transfection to detect the luciferase activity. The recombinant vector pmirGLO-KLF4 (10 ng) and hsa-miR-124-3p mimics, hsa-miR-124-3p NC, hsa-miR-135a-5p mimics, and hsa-miR-135a-5p NC (50 nM) were separately transfected by Lipofectamine 2000 according to the manufacturer's instructions. The empty vector was transfected in a separated group as a control. After 24 hours of culture, the luciferase intensity was detected by the Promega dual-luciferase assay system.

Table 1. Primer sequences for qRT-PCR.

miRNA	Primer sequence (5' to 3')
hsa-miR-124-3p	Forward primer: CGTGTTCACAGCGGACCTTG; Reverse primer: GGCGCCTCTCTTGGCATTC
hsa-miR-135a-5p	Forward primer: GGCCTCGCTGTTCTCTATGG; Reverse primer: GCCACGGCTCCAATCCCTAT
hsa-miR-135b-5p	Forward primer: TGGCCTATGGCTTTTCATTCCT; Reverse primer: AGCTCGCCCCTCACTGTA
hsa-miR-455-5p	Forward primer: GCCTTTGGACTACATCGTGGA; Reverse primer: ACATAGGCCTTGAGGCAAGT
lncRNA MALAT1	Forward primer: ATGCGAGTTGTTCTCCGTCT; Reverse primer: TATCTGCGGTTTCCTCAAGC
β-actin	Forward primer: CATCGTCCACCGCAAATGCTTC; Reverse primer: AACCGACTGCTGTCACCTTCAC

To further verify that *NR3C2* is a target binding gene of hsa-miR-124-3p and hsa-miR-135a-5p, we transfected WT NR3C2, which contains binding sites of hsa-miR-124-3p, WT+hsa-miR-124-3p NC, WT+hsa-miR-124-3p mimic, hsa-miR-124-3p NC, MUT+hsa-miR-124-3p NC, and MUT+hsa-miR-124-3p mimic, as well as WT NR3C2, which contains binding sites of hsa-miR-135a-5p, WT+hsa-miR-135a-5p NC, WT+hsa-miR-135a-5p mimic, hsa-miR-135a-5p NC, MUT+hsa-miR-135a-5p NC, and MUT+hsa-miR-135a-5p mimic, respectively. The luciferase intensities were measured after 24 hours of culture by the Promega dual-luciferase assay system.

Detection of lncRNA *MALAT1* and miRNAs by quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the plasma of patients. HUVECs were cultured with TRIzol LS reagent (Invitrogen, USA). Then the cDNA was produced by the M-MLV reverse transcriptase (Clontech, USA). Quantitative real-time PCR (qRT-PCR) was performed on an ABI 7500 PCR system (Applied Biosystems) using SYBRGreen MasterMix (Bio-Rad, USA). And β-actin was selected as a control to detect relative expression of lncRNA *MALAT1*, hsa-miR-124-3p, hsa-miR-135a-5p, hsa-miR-135b-5p, and hsa-miR-455-5p in plasma and relative expression levels of hsa-miR-124-3p and hsa-miR-135a- 5p in cells. The primers information is shown in Table 1.

Detection of protein expression by western blot

In HUVECs with si-MALAT1, si-MALAT1 NC, or blank control transfection, total protein was extracted. Then a BCA protein assay kit (Thermo Fisher Scientific, Germany) was used to quantify the concentration of the extracted total protein. The lysate

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Table 2. Comparison of general characteristics between hypertensive patients and control subjects.

	Case (n=230)	Control (n 230)	*p*
Age (years, mean±SD)	58.03±8.65	57.56±8.06	0.55
Gender			0.33
Men	153 (66.52%)	143 (62.17%)	
Women	77 (33.48%)	87 (37.83%)	
BMI (kg/m^2, mean±SD)	25.57±2.74	25.09±2.79	0.06
Smoking			<0.01
Ever	120 (52.17%)	73 (31.74%)	
Never	110 (47.83%)	157 (68.26%)	
Drinking			0.03
Ever	102 (44.35%)	79 (34.35%)	
Never	128 (55.65%)	151 (65.65%)	
DBP (mmHg, mean±SD)	93.43±8.84	83.06±9.28	<0.01
SBP (mmHg, mean±SD)	145.00±14.15	136.33±15.89	<0.01
Family history			<0.01
Yes	100 (43.48%)	25 (10.87%)	
No	130 (56.52%)	205 (89.13%)	

BMI – body mass index; DBP – diastolic blood pressure; SBP – systolic blood pressure.

was separated by a 12% SDS-PAGE gel and the separated protein was then transferred to a nitrocellulose membrane. We used 40 μg of the protein sample for the electrophoresis. Western blot analysis was performed using NR3C2 antibody (Rabbit Polyclonal, catalog: 21854-1-AP, Proteintech) and horse radish peroxidase (HRP)-conjugated secondary antibody. The β-actin protein level was measured as an internal control.

Detection of HUVEC apoptosis by Annexin V FITC/PI double staining flow cytometry

After collecting the cells, the concentration of each group was calibrated to 1×10^6/mL and was rinsed with cold phosphate buffer saline and centrifuged. Annexin V FITC (5 μL) and PI (5 μL) were added to 500 μL cell suspension. The mixture was then incubated at 4°C for 30 minutes in the dark and analyzed using a NovoCyte™ 2060 flow cytometer (ACEA Biosciences, USA).

Statistical analyses

The statistical analyses in this study were performed using SPSS v22.0. The continuous variables in this study were showed as mean±standard deviation (SD). One-way ANOVA or *t*-test was performed to compare the differences between groups. The categorical variables were showed as n%, and chi-squared test was performed to analyze the differences between groups. Spearman correlation coefficient was used to evaluate the correlation between lncRNA *MALAT1* and hsa-miR-124-3p, hsa-miR-135a-5p levels, as well as the levels of hsa-miR-124-3p, hsa-miR-135a-5p and NR3C2 protein. A statistical significance was considered when $P<0.05$.

Results

General characteristics of subjects

A total of 230 hypertensive patients and 230 healthy non-hypertensive subjects were selected for the study. No significant difference in age, gender, and body mass index (BMI) was observed between hypertensive patients and healthy subjects ($P>0.05$), as shown in Table 2. Among patients with hypertension, the proportion of patients with smoking, drinking, and family history of hypertension were significant increased compared to that of control study participants ($P<0.05$). Moreover, DBP and SBP of hypertensive patients were also significantly enhanced compared to that in the control participants ($P<0.05$).

Inhibited expression of hsa-miR-124-3p, hsa-miR-135a-5p, and upregulation of lncRNA *MALAT1* in hypertensive patients

We performed qRT-PCR to detect the plasma levels of hsa-miR-124-3p, hsa-miR-135a-5p, hsa-miR-135b-5p, hsa-miR-455-5p, and lncRNA *MALAT1* in all participants. It was revealed that there was a significant decrease in the plasma level of

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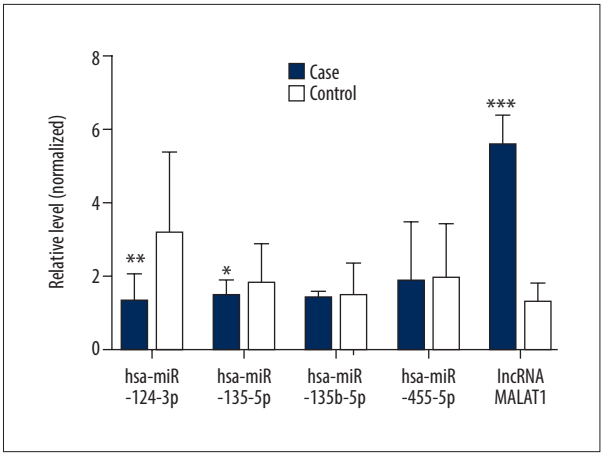

Figure 1. Comparison of the plasma levels of hsa-miR-124-3p, hsa-miR-135a-5p, hsa-miR-135b-5p, hsa-miR-455-5p, and lncRNA *MALAT1* between hypertensive patients and controls. * *P*<0.05, ** *P*<0.01, *** *P*<0.001. lncRNA – long noncoding RNA; MALAT1 – metastasis associated lung adenocarcinoma transcript 1.

hsa-miR-124-3p and hsa-miR-135a-5p in hypertensive patients compared to controls. While we also detected a significant increase in the level of lncRNA *MALAT1* in patients with hypertension compared to controls (*P*<0.05). Furthermore, there were no considerable differences in the plasma levels of hsa-miR-135b-5p and hsa-miR-455-5p among hypertensive patients and healthy study participants (*P*>0.05; Figure 1).

A negative correlation of lncRNA *MALAT1* with hsa-miR-124-3p and hsa-miR-135a-5p

We also found that there was a negative correlation of lncRNA *MALAT1* with hsa-miR-124-3p and hsa-miR-135a-5p in both hypertensive patients and healthy controls (r=–0.64, –0.72, –0.80, –0.77; *P*<0.01, *P*<0.01, *P*<0.01, *P*<0.01, respectively; Figure 2A, 2B, 2E, 2F). However, lncRNA *MALAT1* levels were not significantly correlated with hsa-miR-135b-5p and hsa-miR-455-5p levels (*P*>0.05; Figure 2C, 2D).

A negative correlation of NR3C2 protein with hsa-miR-124-3p and hsa-miR-135a-5p

After analyzing the plasma levels of NR3C2 protein, we revealed an upregulation of NR3C2 protein in the plasma of hypertensive patients compared to that of controls (*P*<0.01; Figure 3A). We further analyzed the correlation between plasma miRNA and NR3C2 protein in hypertensive patients, and found a negative correlation of NR3C2 protein with hsa-miR-124-3p and hsa-miR-135a-5p (r=–0.74, –0.84; *P*<0.01, *P*<0.01, respectively; Figure 3B, 3C). However, there was no significant correlations between hsa-miR-135b-5p, hsa-miR-455-5p levels, and NR3C2 protein expression level (*P*>0.05; Figure 3D, 3E).

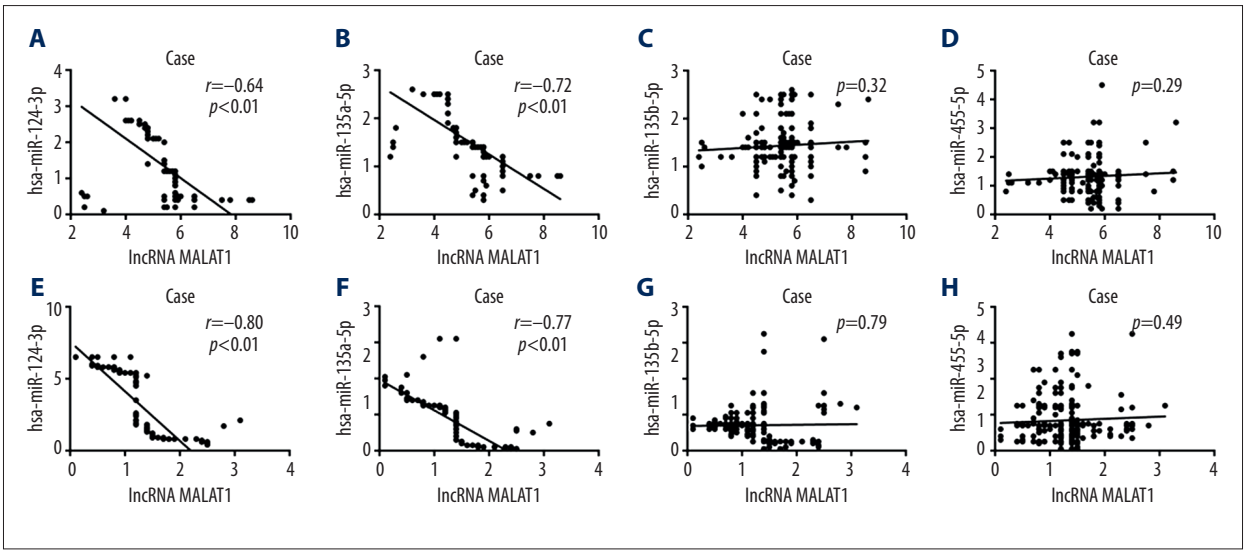

Figure 2. Correlation of lncRNA *MALAT1* with the levels of miRNAs in the plasma. (**A–D**) Correlation observed between the levels of hsa-miR-124-3p, hsa-miR-135a-5p, hsa-miR-135b-5p, hsa-miR-455-5p, and lncRNA *MALAT1* in hypertensive patients. (**E–H**) Correlation observed between the levels of hsa-miR-124-3p, hsa-miR-135a-5p, hsa-miR-135b-5p, hsa-miR-455-5p, and lncRNA *MALAT1* in the control group. lncRNA – long noncoding RNA; MALAT1 – metastasis associated lung adenocarcinoma transcript 1; miRNAs – microRNAs.

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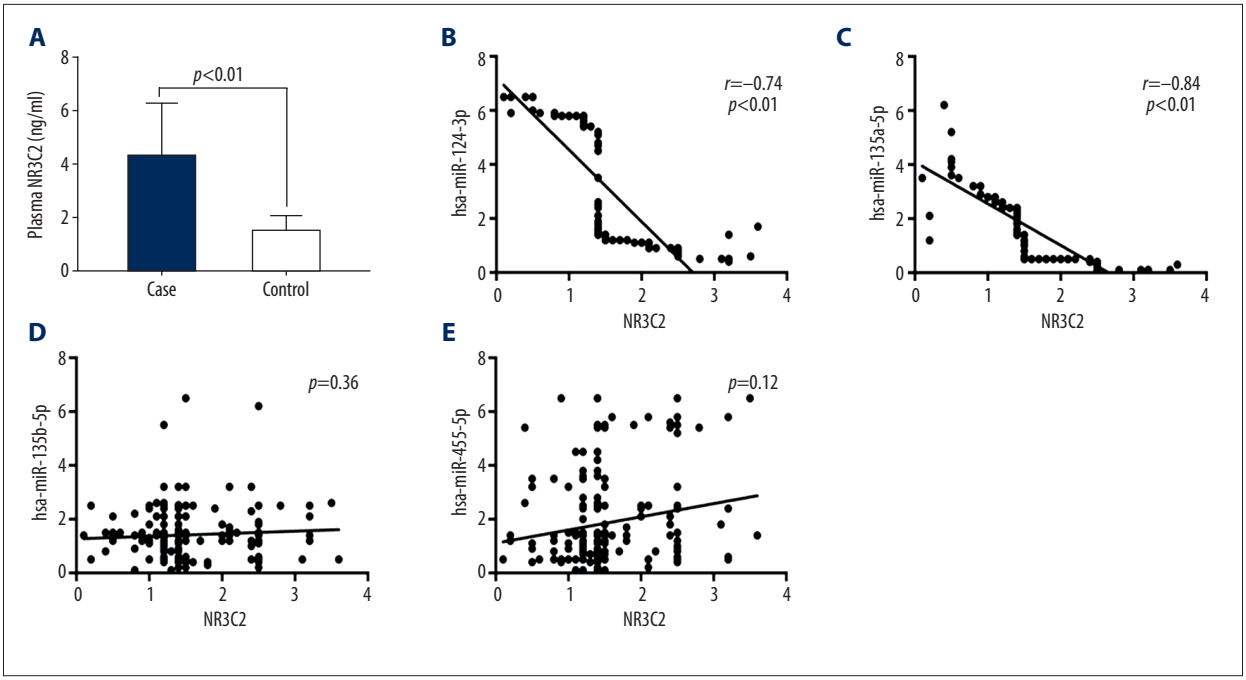

Figure 3. Correlation between NR3C2 protein expression levels and hsa-miR-124-3p, hsa-miR-135a-5p. (**A**) The plasma NR3C2 protein was significantly increased in hypertensive patients. (**B**) A negative correlation of the plasma NR3C2 protein expression level with hsa-miR-124-3p level in patients with hypertension. (**C**) A negative correlation of the plasma NR3C2 protein expression level with hsa-miR-135a-5p in hypertensive patients. (**D**) The plasma NR3C2 protein expression was not associated with hsa-miR-135b-5p in hypertensive patients. (**E**) The plasma NR3C2 protein expression level was not associated with hsa-miR-455-5p in hypertensive patients. NR3C2 – nuclear receptor subfamily 3, group C, member 2.

Regulation of HUVEC proliferation and apoptosis by lncRNA *MALAT1* via the hsa-miR-124-3p/NR3C2 and/or hsa-miR-135a-5p/NR3C2 axis

Through cellular transfection experiments, we found that cell proliferation was significantly inhibited in HUVECs after the inhibition of *MALAT1* (Figure 4A). After knocking down *MALAT1*, the expressions of hsa-miR-124-3p and hsa-miR-135a-5p were significantly elevated in HUVECs (Figure 4B), and the NR3C2 protein level was decreased (Figure 4C). The apoptotic rate of HUVECs after transfection of si-MALAT1 was significantly lower than that of transfected si-MALAT1 NC and blank control group (si-MALAT1: 3.64±0.21% *vs.* si-MALAT1 NC: 3.76±0.19%, blank control: 10.51±1.24%; Figure 4D).

LncRNA *MALAT1* was served as a molecular sponge for hsa-miR-124-3p and hsa-miR-135a-5p

By using an online prediction tool, starbase (*http://starbase.sysu.edu.cn/agoClipRNA.php?source=lncRNA*), for predicting lncRNA-miRNA interaction, we found that there are binding sites of both hsa-miR-124-3p and hsa-miR-135a-5p in the lncRNA *MALAT1* (Figure 5A, 5B). To further identify whether hsa-miR-124-3p and hsa-miR-135a-5p bind to the predicted sites of *MALAT1*, we constructed WT and MUT MALAT1 luciferase reporter vectors, respectively. After the co-transfection of the hsa-miR-124-3p binding site WT and hsa-miR-124-3p mimic or hsa-miR-135a-5p binding site WT and hsa-miR-135a-5p mimic, but not the hsa-miR-124-3p binding site MUT and the hsa-miR-135a-5p binding site MUT, the luciferase activity in HUVECs was significantly reduced (Figure 5C, 5D).

NR3C2 was acted as a target gene of hsa-miR-124-3p and hsa-miR-135a-5p

To further demonstrate whether hsa-miR-124-3p and hsa-miR-135a-5p could target the 3'UTR of *NR3C2*, we conducted a dual-luciferase reporter gene assay. A fragment of the 3' UTR region of *NR3C2* containing about±50 bp centered at the predicted target site was cloned into the downstream region of the luciferase reporter gene in a reporter vector – pmirGLO – to obtain a WT reporter vector (Figure 6A, 6B). In addition, an MT reporter vector was constructed, and the sequence of the reporter vector was confirmed by sequencing. Co-transfection of hsa-miR-124-3p mimic and a reporter vector containing the 3-UTR of *NR3C2*, hsa-miR-135a-5p mimic containing the 3'UTR of *NR3C2* reporter vector into HEK 293T cells was performed, and the effects of hsa-miR-124-3p and hsa-miR-135a-5p on the luciferase activity of *NR3C2* were detected using the dual fluorescence reporter system. We revealed that after the

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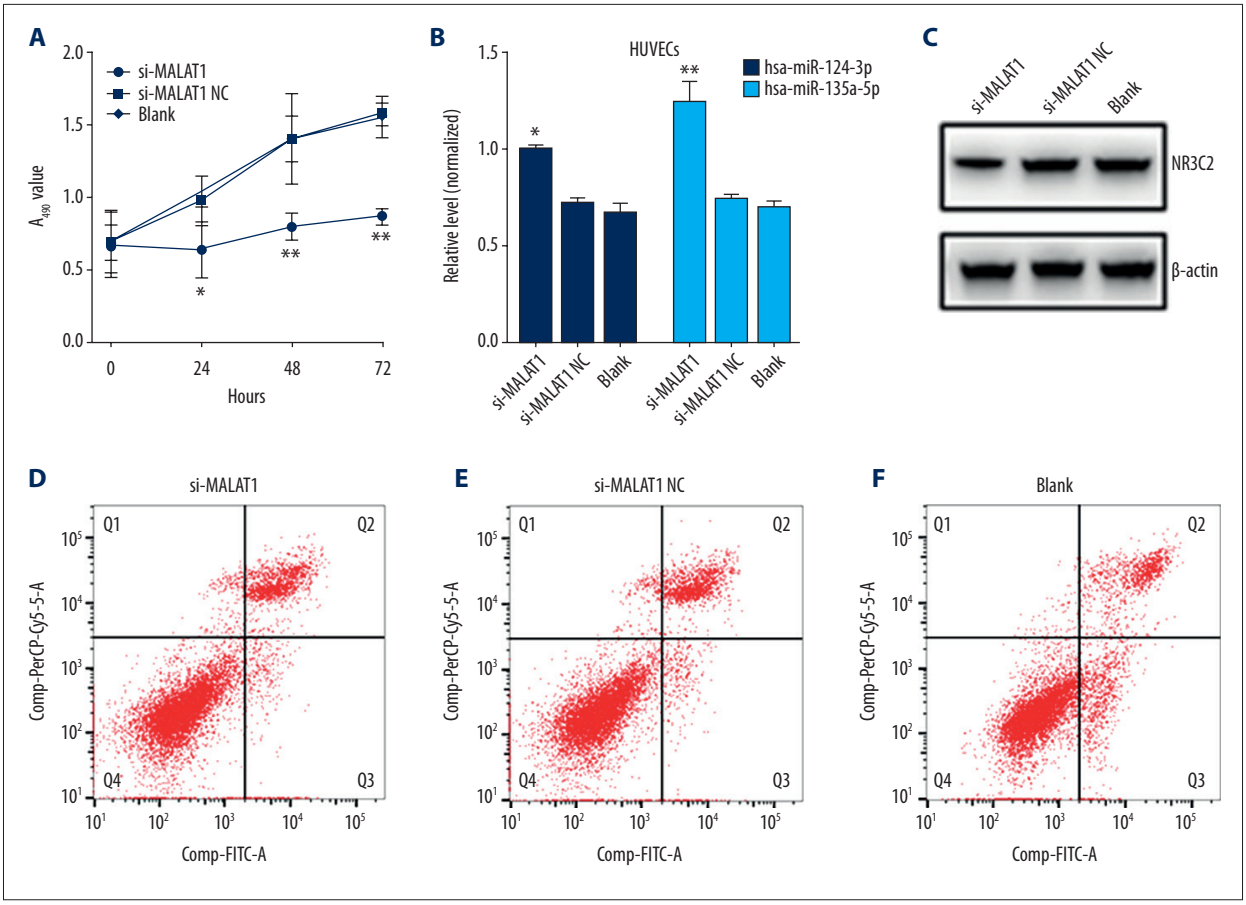

Figure 4. Results of cell transfection experiments. (**A**) Detecting the proliferation of cells after transfection by CCK-8. (**B**) Detection of hsa-miR-124-3p and hsa-miR-135a-5p levels by qRT-PCR, compared to the blank group, * $P<0.05$, ** $P<0.01$. (**C**) Detecting the levels of NR3C2 protein in HUVECs after transfection and confirmation by western blot. (**D–F**) The apoptosis rate of HUVECs after transfection of si-MALAT1 (**D**), si-MALAT1 NC (**E**) and blank control (**F**). CCK-8 – Cell Counting Kit-8; qRT-PCR – quantitative real-time polymerase chain reaction; HUVECs – human umbilical vein endothelial cells; MALAT1 – metastasis associated lung adenocarcinoma transcript 1; NR3C2 – nuclear receptor subfamily 3, group C, member 2.

upregulation of hsa-miR-124-3p and hsa-miR-135a-5p and the luciferase activity of the corresponding WT reporter vector was reduced, while the MT reporter vector had no significant changes on the luciferase activity (Figure 6C, 6D).

Discussion

In the past years, China's economy has experienced rapid growth, and along with the changes in lifestyle and diet structure, cardiovascular diseases have become the leading cause of death in China. Hypertension is a "cardiovascular syndrome" characterized by a sustained increase in arterial blood pressure and has been recognized as an essential risk factor for cardiovascular diseases and other vascular diseases such as heart failure, coronary heart disease, peripheral vascular disease, stroke, and chronic kidney disease [24,25]. Demonstrated previous study has demonstrated that even the slightest increase in blood pressure (>115/70 mmHg), can increase the risk of cardiovascular disease [26]. Thus, early prevention and treatment of hypertension are of great significance for improving the prognosis of such patients. Endothelial dysfunction is considered an early pathophysiological feature of hypertension. In hypertensive states, high pressure in the vascular cavity results in the activation of endothelial cells, release of inflammation and procoagulant factors, and adhesion of neutrophils and platelets. Endothelial cells not only have a substantial influence on vascular remodeling via abnormal proliferation and apoptosis, but also on the pathological migration of vascular endothelial cells that leads to changes in vascular structure, thereby initiating and maintaining hypertension and vascular remodeling [27]. Moreover, endothelial cell dysfunction is more closely related to organ damage and clinical diagnosis of patients with hypertension. Hence, it is critical to investigate the development of hypertensive vascular endothelial injury for the prevention as well as treatment of hypertension. To date,

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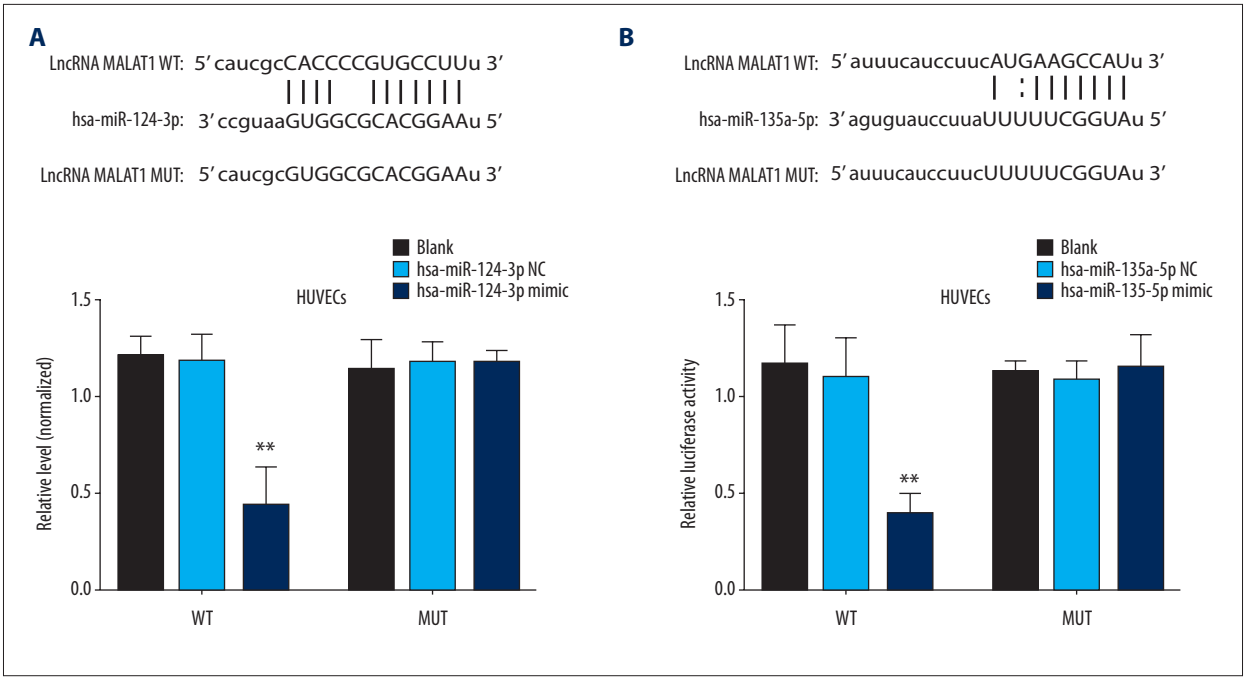

Figure 5. Results of the dual-luciferase reporter gene assay. (**A**) The hsa-miR-124-3p interacted with lncRNA *MALAT1* at the predicted site in HUVECs. (**B**) The hsa-miR-135-5p interacted with lncRNA *MALAT1* at the predicted site in HUVECs. ** $P<0.01$, compared to the blank control group. lncRNA – long noncoding RNA; MALAT1 – metastasis associated lung adenocarcinoma transcript 1; HUVECs – human umbilical vein endothelial cells.

its underlying molecular mechanism has not been completely elucidated and requires to be further studied.

The role of lncRNA in cardiovascular diseases has become an important topic of research. The expression of lncRNA is elevated not only in many types of tumor cells but also is in endothelial cells [28–30]. Although *MALAT1* is involved in diverse pathways, the ceRNA hypothesis offers a new direction for the control of gene expression [31]. Previous studies showed that miRNAs are negatively correlated with *MALAT1* [30,32], and the binding of miRNAs to *MALAT1* reduces the level of miRNAs, accompanied by an increased expression of miRNA targeted genes [33]. Zhu et al. [34] found that MALAT1 can inhibit H/R-stimulated HUVEC damage by targeting the miR-320a/RAC1 axis. Li et al. [35] showed that lncRNA MALAT1 contributed essentially to hypertension. The current study revealed that *MALAT1* serves as a miRNAs molecular sponge. Here, a negative association of *MALAT1* levels with the plasma levels of hsa-miR-124-3p and hsa-miR-135a-5p was found in hypertensive patients. Meanwhile, dual-luciferase reporter gene experiments showed that *MALAT1* can also target hsa-miR-124-3p and hsa-miR-135a-5p. It was shown that *MALAT1* acts as an endogenous modulator via directly targeting miR-124 [36].

In the cell transfection experiments, we found that the proliferation of HUVECs was markedly reduced, and the apoptosis level of HUVECs was significantly increased after the downregulation of *MALAT1*. After the knock-down of *MALAT1*, the levels of hsa-miR-124-3p and hsa-miR-135a-5p in HUVECs were elevated, and the expression level of NR3C2 protein was significantly decreased, suggesting that *MALAT1* can downregulate the expression level of hsa-miR-124-3p and hsa-miR-135a-5p. Meanwhile, *MALAT1* also upregulates the expression level of NR3C2 protein.

The *nuclear receptor subfamily 3, group C, member 2* (*NR3C2*) gene is located on chromosome 4q31.23 and plays an important role in the progress of hypertension [20,37]. In the present study, using the Targetscan tool, we revealed that *NR3C2* was a target gene of hsa-miR-124-3p and hsa-miR-135a-5p. This was also confirmed using dual-luciferase reporter assays. Moreover, it was showed that hsa-miR-124-3p and hsa-miR-135a-5p reduce *NR3C2* expression and regulate the renin-angiotensin-aldosterone system [20].

However, some limitations restricted the objectives of this study. First, this study needs to be further validated in animal models. Second, we could not exclude the possibility that other miRNAs also contributed to *NR3C2* expression, since their roles were blocked by endogenous miRNAs.

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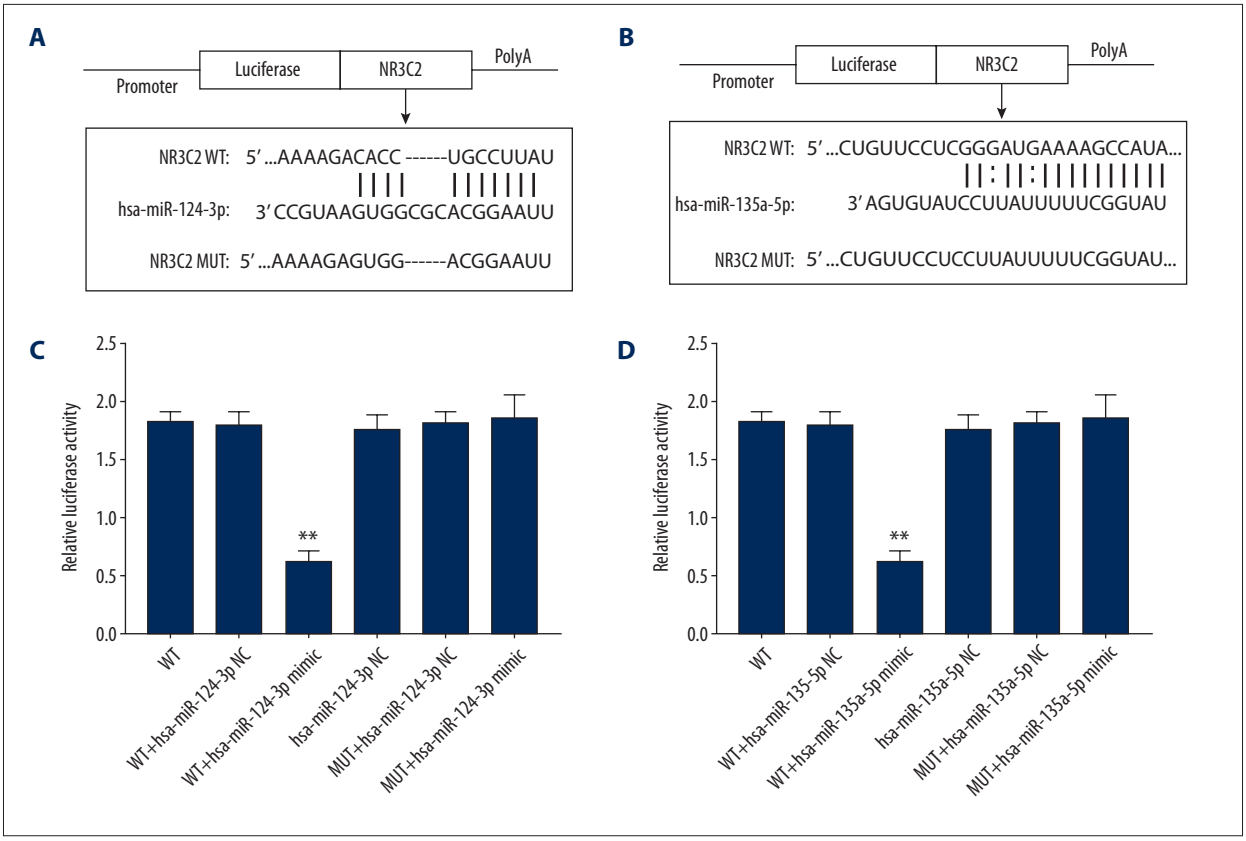

Figure 6. Outcomes of dual luciferase reporter gene assay. (**A, C**) *NR3C2* is the target gene of hsa-miR-124-3p, ** $P<0.01$, compared to the WT. (**B, D**) *NR3C2* is the target gene of hsa-miR-135a-5p, ** $P<0.01$, compared to the WT. NR3C2 – nuclear receptor subfamily 3, group C, member 2; WT – wild type.

Conclusions

Bioinformatics screening revealed that a variety of miRNAs may serve as molecular sponges of *MALAT1*; therefore, we selected the 2 miRNAs, hsa-miR-124-3p and hsa-miR-135a-5p. We further demonstrated that the 3'UTR of *NR3C2* was located at the binding sites of hsa-miR-124-3p and hsa-miR-135a-5p. We showed that lncRNA *MALAT1* might control the proliferation and apoptosis of HUVECs via hsa-miR-124-3p/NR3C2 and/or hsa-miR-135a-5p/NR3C2 axis. However, further studies in animal models are necessary to further validate whether the regulation of lncRNA *MALAT1* through the hsa-miR-124-3p/NR3C2 and/or hsa-miR-135a-5p/NR3C2 axis is relevant to the development of hypertension.

Conflicts of interest

None.

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