Long Non-Coding RNA MALAT1 Promotes Acute Cerebral Infarction Through miRNAs-Mediated hs-CRP Regulation

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Abstract

The occurrence of cerebral infarction commonly takes atherosclerosis as the pathophysiological basis, accompanied by chronic inflammation. Hypersensitive C-reactive protein (hs-CRP) is an important inflammatory factor involved in the formation of atherosclerosis. This study aims to investigate the regulation of hs-CRP expression by long-chain non-coding RNA (LncRNA) MALAT1 in acute cerebral infarction patients. Plasma levels of LncRNA MALAT1 and hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in 256 Chinese Han ACI patients and 256 controls were analyzed. HUVECs were transfected with LncRNA MALAT1, MALAT1 NC, and si-MALAT1, respectively. The expression levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p were analyzed. Then, HUVECs were transfected with hsa-miR-145-5p inhibitor, hsa-miR-140-5p inhibitor, hsa-miR-483-3p inhibitor, hsa-miR-338-3p inhibitor, and hsa-miR-145-5p mimic, hsa-miR-140-5p mimic, hsa-miR-483-3p mimic, hsa-miR-338-3p mimic, and the expression level of hs-CRP was detected by Western blotting. The levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in the plasma of ACI patients were significantly lower than those in the control group ($p < 0.001$), and the plasma LncRNA MALAT1 levels were significantly higher in ACI patients than in the control group ($p < 0.001$). The level of LncRNA MALAT1 in plasma of ACI patients and control group was negatively correlated with hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p ($r = -0.36, -0.79, -0.76, -0.75; -0.60, -0.68, -0.48, -0.56$). Plasma levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p were negatively correlated with hs-CRP levels in patients with ACI and controls ($r = -0.74, -0.81, -0.84, -0.56; -0.61, -0.69, -0.69, -0.50$). MALAT1 transfection resulted in the decreased levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in HUVECs while overexpression of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p led to a decrease in hs-CRP levels in HUVECs. LncRNA MALAT1 induced the upregulation of CRP expression through inhibiting the expression of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p.

Keywords Acute cerebral infarction · Long-chain non-coding RNA · High-sensitivity C-reactive protein · microRNAs

Introduction

Acute cerebral infarction (ACI) is a common cerebrovascular disease with increasing morbidity, disability, and mortality (Bielewicz et al. 2010; Murray and Lopez 2013). Notably, due to the life pressure and unhealthy lifestyles, the incidence of mid-cerebral infarction in young people is growing continuously (Wong et al. 2013; Randall et al. 2016). The incidence of cerebral infarction in the Chinese population ranks among the top in the world, and cerebrovascular disease has already become the leading cause of death in the Chinese population (Zhang et al. 2015; Chang et al. 2017; Yang et al. 2013). The occurrence of ACI is affected by multiple factors such as genetic factors and environment. But, its pathogenesis, diagnostic methods, and prognostic criteria have not been fully elucidated yet (Ma et al. 2006; Tang et al. 2017).

MicroRNAs (miRNAs) are a class of single-strand and small non-coding RNAs (containing about 22 nucleotides) that can inhibit the biological function of target genes by binding to the 3′ untranslated region (UTR) of the target gene mRNA (Mohr and Mott 2015). MicroRNAs are widely present in living individuals and are involved in the occurrence and progression of most diseases such as cerebrovascular

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diseases (Volny et al. 2015) and inflammatory diseases (Xiaoyan et al. 2017; Schaefer 2016; Soroosh et al. 2018).

Long non-coding RNAs (lncRNAs) are a type of RNA with lengths exceeding 200 nucleotides that are not translated into protein (Spizzo et al. 2012). LncRNAs play an important role in many life activities including dose compensation, epigenetic regulation, cell-cycle regulation, and cell differentiation regulation (Wapinski and Chang 2011; Xiao et al. 2009; Gupta et al. 2010; Mercer and Mattick 2013; Tripathi et al. 2013; Hu et al. 2013). LncRNAs expression is tissue specific in humans, and its expression profile and expression abundance vary widely among tissues. Current studies on the relationship between LncRNA and disease mainly focus on tumors and neurodegenerative diseases (Clark and Blackshaw 2014; Runge et al. 2014). Recent studies have shown that stroke can significantly alter the expression of mRNA and non-coding RNA in the brain (Vemuganti 2013) and cerebral infarction can also induce changes of the LncRNA expression profile in the brain (Dharap et al. 2012). These findings suggest that some certain LncRNAs may play a critical role in the pathology of acute cerebral infarction.

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), also known as NEAT2 (non-coding nuclear-enriched abundant transcript 2), is a multi-functional lncRNA which is widely expressed in a variety of tumors and tissues (Huang et al. 2016). Previous studies demonstrated MALAT1 is capable of specifically recruiting members of the SR protein family and is involved in epigenetic regulation (Cho et al. 2014), cell-cycle regulation (Tripathi et al. 2013), and angiogenesis (Tee et al. 2016). However, there are few researches on the role of MALAT1 in the development and progression of ACI.

C-reactive protein (CRP), firstly identified in the 1930s, is an acute-phase protein synthesized by hepatocytes when the body is subjected to inflammatory stimulation such as microbial invasion or tissue damage (Hind et al. 1985). Human CRP scavenges necrotic and apoptotic cells and pathogens through activating the complement and mononuclear phagocytic system after binding to its ligand. Elevated serum levels of C-reactive protein (CRP) are found in up to three-quarters of patients with ACI, indicating CRP can function as one potential prognostic biomarker (Ridker et al. 1998; Smith et al. 2006). However, the mechanism by which CRP is upregulated in ACI patients remains unclear.

In this study, four miRNAs that can simultaneously bind the 3′UTR region of CRP gene and MALAT1 were found by using TargetScan (http://www.targetscan.org/mamm_31/) and starbase databases (http://starbase.sysu.edu.cn/agoClipRNA.php?source=lncRNA), namely hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483- 3p, and hsa-miR-338-3p (Table 1 and Fig. 1). Functional experiments confirmed that LncRNA MALAT1 participates in regulating the expression of hs-CRP through miRNAs.

Table 1 Binding site information of CRP gene 3′UTR region-miRNAs-LncRNA MALAT1

| #	CRP gene 3′UTR	miRNAs	LncRNA MALAT1
1	1070-1105 bp	hsa-miR-145-5p	chr11:65267120-65267142
2	832-862 bp	hsa-miR-140-5p	chr11:65272388-65272412
			chr11:65272945-65272966
			chr11:65265449-65265469
3	961-993 bp	hsa-miR-483-3p	chr11:65269083-65269105
4	195-220 bp	hsa-miR-338-3p	chr11:65273302-65273323
			chr11:65272551-65272575
			chr11:65273574-65273596

Materials and Methods

Subjects

A total of 256 Chinese Han ACI patients over 18 years old who were treated in the Shanghai East Hospital from August 2015 to August 2018 were selected in the study, including 161 males and 95 females. According to the 2013 AHA/ASA guidelines for the diagnosis of ACI (Deguchi et al. 2006), all patients underwent brain CT scans or MRI to exclude patients with other intracranial lesions, such as tumors and infections. The collected subjects had been diagnosed with acute cerebral infarction and received an initial brain MRI within 72 h of admission. Patients with the following conditions were eliminated: (1) patients who had recently undergone vascular stenting; (2) patients with heart valve replacement and pacemaker placement; and (3) patients who were unable to undergo MRI.

Another 256 healthy individuals (163 males and 93 females) were recruited as a control group. General clinical data were collected for all subjects, including age, gender, body mass index (BMI), smoking, alcohol consumption, hypertension, diabetes, and the proportion of patients with hyperlipidemia. The experimental scheme was approved by the Ethics Committee of Shanghai East Hospital, and all patients and healthy individuals signed informed consent forms.

Cell Culture

Primary human umbilical vein endothelial cells (HUVECs) were maintained in M199 medium supplemented with 20 mg/ml ECGS (Upstate Biotechnology, Lake Placid, NY), 10% FBS (Hyclone, Logan, UT), and 1% penicillin-streptomycin (Thermo Fisher Scientific, Waltham, MA). Cells were cultured in a humidified atmosphere containing 5% CO_2 at 37 °C.
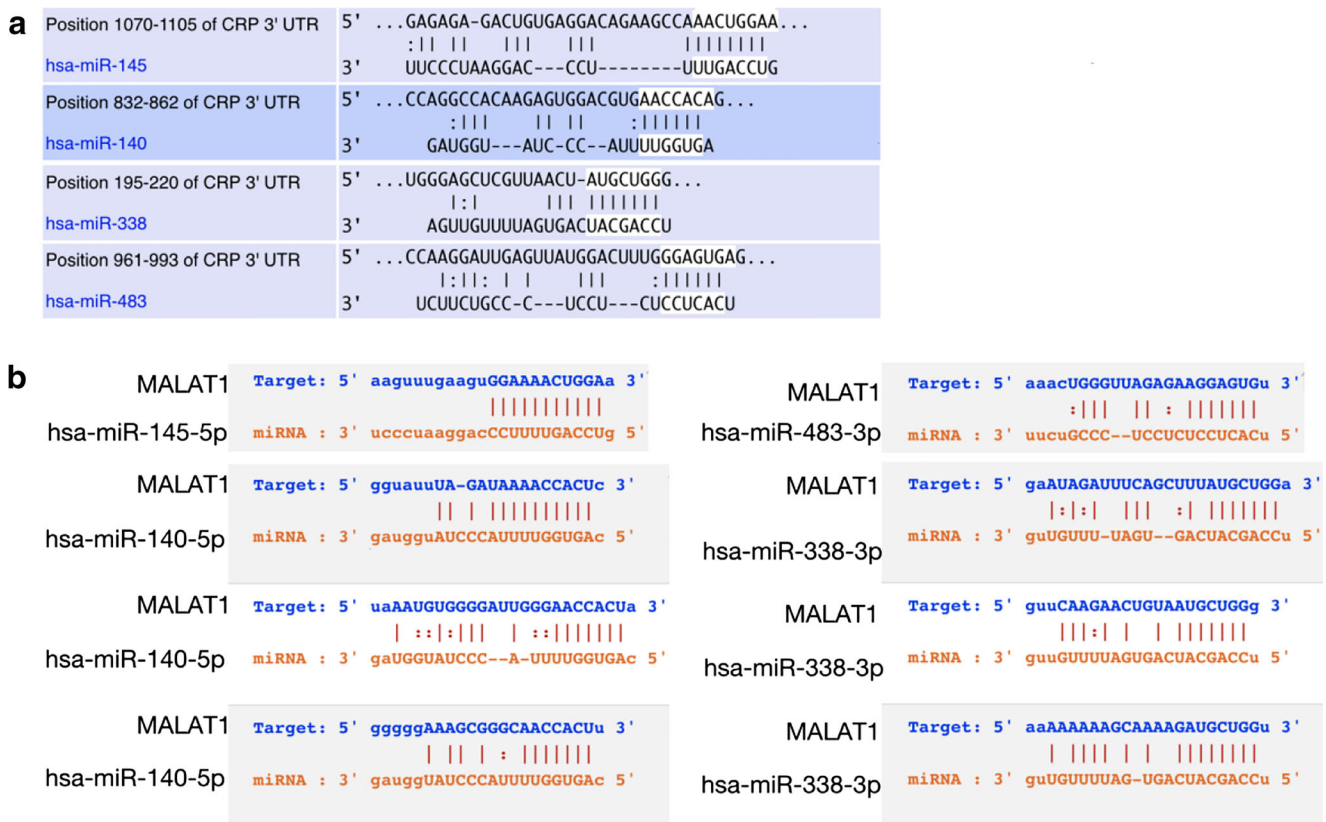
Fig. 1 Prediction of RNA binding sites. **a** Prediction of binding sites between CRP gene and miRNAs. **b** Prediction of binding sites between MALAT1 and miRNAs

siRNA Transfection

All the synthetic non-coding RNAs in this study were purchased from Shanghai GenePharma (Shanghai, China). HUVECs were transfected with siRNA by using Lipofectamine TM RNAiMAX (Life Technologies, Grand Island, NY) according to the user's manual. All the cells were harvested for RNA and protein expression analysis at 48 h post-transfection. The sequences of MALAT1-siRNA and MALAT1-si-NC were 3′-dTdTU CCU UGG UGA AUU GAU AAG TA-5′ and 5′-GAU AUC UGC AGU UGC UAA A-3′. The sequences of hsa-miR-145-5p inhibitor and hsa-miR-145-5p mimic were 5′-AGG GAU UCC UGG GAA AAC UGG AC-3′ and 5′-GUC CAG UUU UCC CAG GAA UCC CU-3′. The sequences of hsa-miR-140-5p inhibitor and hsa-miR-140-5p mimic were 5′-CUA CCA UAG GGU AAA ACC ACU G-3′ and 5′-CAG UGG UUU UAC CCU AUG GUA G-3′. Sequences for hsa-miR-483-3p inhibitor and hsa-miR-483-3p mimic were 5′-AAG ACG GGA GGA GAG GAG UGA-3′ and 5′-UCA CUC CUC UCC UCC CGU CUU-3′. Sequences for hsa-miR-338-3p inhibitor and hsa-miR-338-3p mimic were 5′-GCA AAA AUU AGU GUG CGC CAA A-3′ and 5′-UCC AGC AUC AGU GAU UUU GUU G-3′. The sequence of RNA oligomers used as miR-NC was 5′-AAA UGU ACU GCG CGU GGA GAC-3′.

RNA Isolation and Quantitative RT-PCR

Total RNA was extracted by TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The reverse transcription reaction of 1 μg RNA was carried out by using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). Quantitative RT-PCR was performed by FastStart Universal SYBR-Green Master (Roche, Indianapolis, IN). The primer sequences for RT-PCR were as follows: 5′-GGG GTA CCG CTA GCA GAG CAA TAA GCC ACA TCC G-3′(sense) and 5′-CCG CTC GAG TTA CCT CCA GGG ACA GCC TTC-3′(antisense) for hsa-miR-145-5p; 5′-TGG TGT GTG GTT CTA TGC CAG C-3′(sense) and 5′-AGC CTC AAG CCA GAA TTC AGG-3′(antisense) for hsa-miR-140-5p; 5′-TGC GGT CCA GCA TCA GTG AT-3′(sense) and 5′-CCA GTG CAG GGT CCG AGG T-3′(antisense) for hsa-miR-338-3p; 5′-TGC GGG TGC TCG CTT CGG CAG C-3′(sense) and 5′-CCA GTG CAG GGT CCG AGG T-3′(antisense) for U6. The expression of U6 was detected as the endogenous control, and all the samples were normalized to U6 following the $2^{-\Delta\Delta CT}$ method.

Western Blotting Assay

Total protein was extracted from HUVECs (about 1×10^7) using cell lysate containing 1% PMSF (Beyotime Institute of Biotechnology, Haimen, China). The total protein concentration was determined by BCA Protein Assay Kit (Tiangen Biotech Co., Ltd., Beijing, China). Isolated proteins were separated by 10% SDS-PAGE (loading 30 μg per lane) and transferred onto PVDF membranes (EMD Millipore, Billerica, MA, USA). Then, the membrane was blocked with 5% BSA for 1 h and then incubated with the polyclonal CRP antibody (Abeam, Cambridge, UK) (1:10000) and β-actin antibody (Sigma, St. Louis, MS) for 2 h at room temperature. After thrice washing in $1 \times$ TBST, the membrane was incubated with corresponding HRP-labeled secondary antibodies (Abcam, Cambridge, UK) at room temperature for 2 h. Protein bands were visualized using an enhanced chemiluminescence kit (Beyotime Institute of Biotechnology, Haimen, China).

Detection of Plasma hs-CRP Protein Level

The plasma hs-CRP protein level was quantified by Turbidimetric inhibition immuno assay (Kanto Chemical Co Inc., Tokyo, Japan). The instrument was a HITACHI 7600 automatic analyzer (Hitachi Ltd., Tokyo, Japan), and all operations were strictly carried out in accordance with the specification of the supplier. The normal reference value ranged from 0.07 to 500 mg/L.

Statistical Analysis

Statistical analysis was performed using SPSS software of version 21.0 (SPSS Inc., Chicago, IL). The continuous variables were represented as mean ± standard deviation and Student's t test or one-way analysis were used for statistical difference detection. The categorical variable was expressed n (%), and the chi-square test was used for statistical analysis. The correlation between LncRNA MALAT1 and miRNA, miRNAs, and plasma hs-CRP protein levels was analyzed by Pearson correlation. p value less than 0.05 was considered statistically significant.

Results

The Information of ACI Patients and Healthy Control

The general information of the enrolled 256 ACI patients and 256 healthy individuals in the study was presented in Table 2. There was no statistically significant difference in age, gender distribution, BMI, smoking, and alcohol consumption between the ACI patients and the control group

Table 2 Comparison of general information between ACI patients and the control group

Characteristic	ACI ($n = 256$)	Control ($n = 256$)	p
Age (mean ± SD)	58.91 ± 9.63	59.54 ± 10.35	0.48
Gender (n (%))			
Male	161 (62.89%)	163 (63.67%)	0.85
Female	95 (37.11%)	93 (36.33%)	
BMI (kg/m^2, mean ± SD)	25.36 ± 2.15	25.42 ± 2.24	0.76
Smoking (n (%))			
Yes	85 (33.20%)	81 (31.64%)	0.71
No	171 (66.80%)	175 (68.36%)	
Drinking (n (%))			
Yes	94 (36.72%)	101 (39.45%)	0.52
No	162 (63.28%)	155 (60.55%)	
Hypertension (n (%))			
Yes	138 (53.91%)	65 (25.39%)	< 0.001
No	118 (46.09%)	191 (74.61%)	
Diabetes (n (%))			
Yes	64 (25.00%)	31 (12.11%)	< 0.001
No	192 (75.00%)	225 (87.89%)	
Hyperlipidemia (n (%))			
Yes	31 (12.11%)	15 (5.86%)	0.01
No	225 (87.89%)	241 (94.14%)	

ACI, acute cerebral infarction; *SD*, standard deviation; *BMI*, body mass index

($p > 0.05$). The percentage of patients with hypertension, diabetes, and hyperlipidemia in the ACI group was significantly higher than that in the control group, and the difference was statistically significant.

Detection of miRNA Level in Plasma

The levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in plasma of all the subjects were detected by RT-PCR. As shown in Fig. 2, for both male and female subjects, the levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in the plasma of ACI patients were significantly lower than those in the control group ($p < 0.001$).
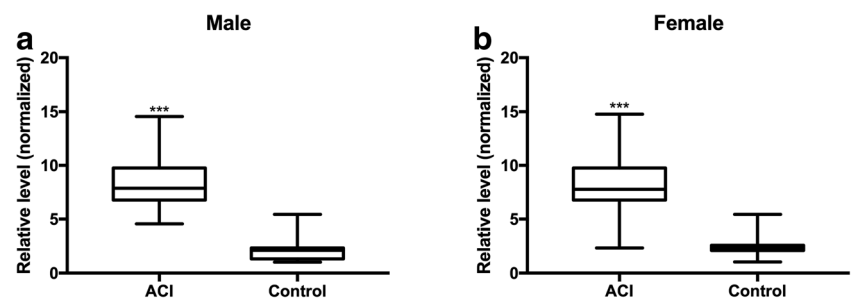
The Level of MALAT1 Negatively Correlated with miRNA Levels in Plasma

The plasma MALAT1 level of both male and female subjects was measured by RT-qPCR. The results showed that the level of MALAT1 in ACI patients was significantly higher than that of the control group ($p < 0.001$) (Fig. 3). The correlation analysis showed that the level of MALAT1 in plasma of ACI patients and the control

Fig. 2 Comparison of plasma miRNA level between ACI patients and the control group. ACI, acute cerebral infarction; ***$p < 0.001$, compared with control
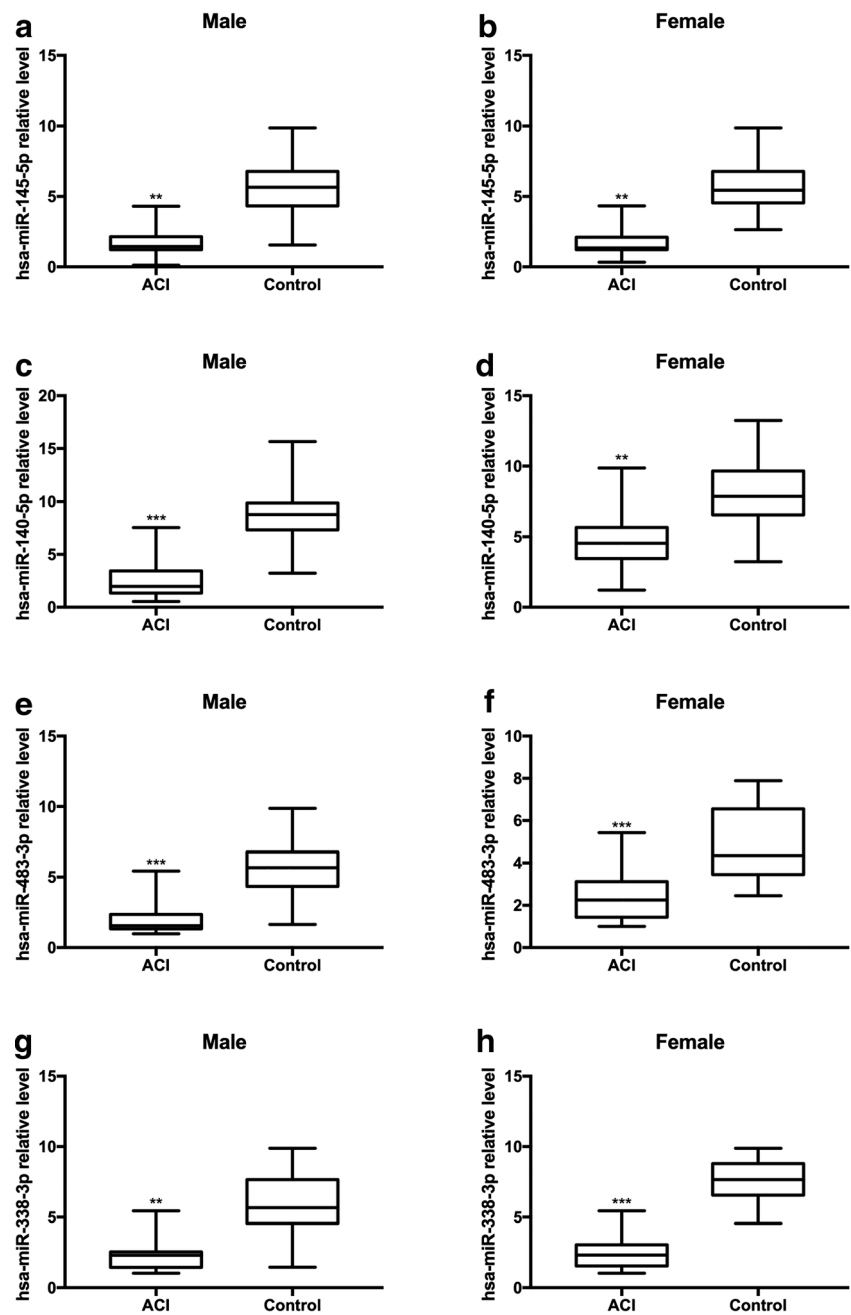
Fig. 3 Comparison of plasma MALAT1 levels between ACI patients and the control groups. **a** Male subjects. **b** Female subjects. ACI, acute cerebral infarction; ***$p < 0.001$, compared with control

group was negatively correlated with hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in plasma of ACI patients and the control group ($r = -0.36, -0.79, -0.76, -0.75; -0.60, -0.68, -0.48, -0.56$) (Fig. 4).

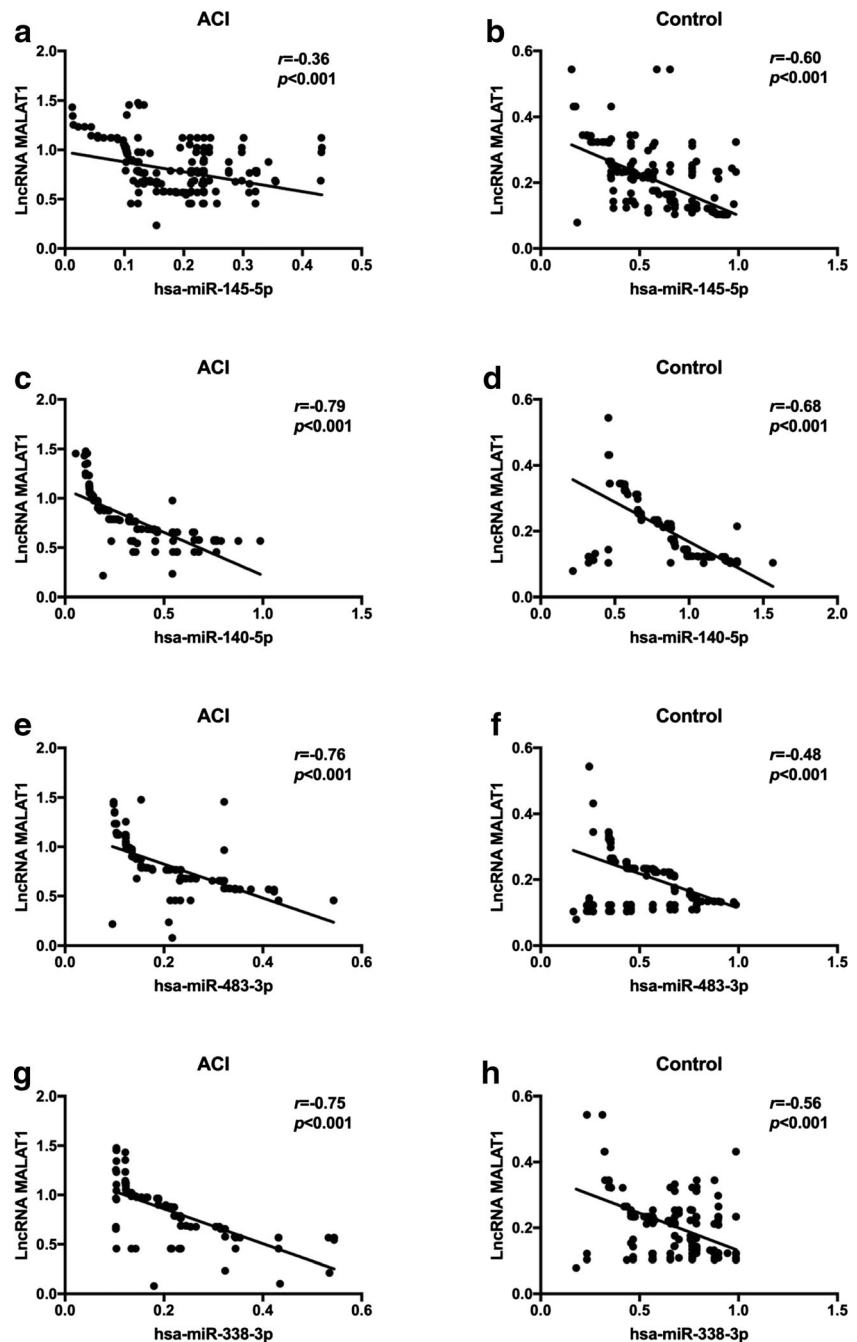## Correlation Between miRNA Levels and Hs-CRP Levels in Plasma

As shown in Fig. 5, the correlation analysis showed that the plasma levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p were negatively correlated with hs-CRP levels in ACI patients and controls ($r = -0.74, -0.81, -0.84, -0.56; -0.61, -0.69, -0.69, -0.50$).

MALAT1 Negatively Regulates miRNAs Expression

The MALAT1 level in plasma was negatively correlated with the level of miRNAs, indicating MALAT1 may have an effect on the expression of miRNAs. In order to investigate the relationship between MALAT1 and miRNAs, MALAT1, MALAT1-NC, si-MALAT1, and si-NC were transfected into

Fig. 4 Correlation between MALAT1 levels and miRNA levels in plasma. The correlations between plasma MALAT1 levels and hsa-miR-145-5p (a), hsa-miR-140-5p (c), hsa-miR-483-3p (e), hsa-miR-338-3p (g) levels in ACI patients. The correlations between plasma LncRNA MALAT1 levels and hsa-miR-145-5p (b), hsa-miR-140-5p (d), hsa-miR-483-3p (f), hsa-miR-338-3p (h) levels in the control group

Fig. 5 Correlation between hs-CRP levels and miRNA levels in plasma. The correlations between plasma hs-CRP levels and hsa-miR-145-5p (**a**), hsa-miR-140-5p (**c**), hsa-miR-483-3p (**e**), hsa-miR-338-3p (**g**) levels in ACI patients. The correlations between plasma hs-CRP levels and hsa-miR-145-5p (**b**), hsa-miR-140-5p (**d**), hsa-miR-483-3p (**f**), hsa-miR-338-3p (**h**) levels in the control group
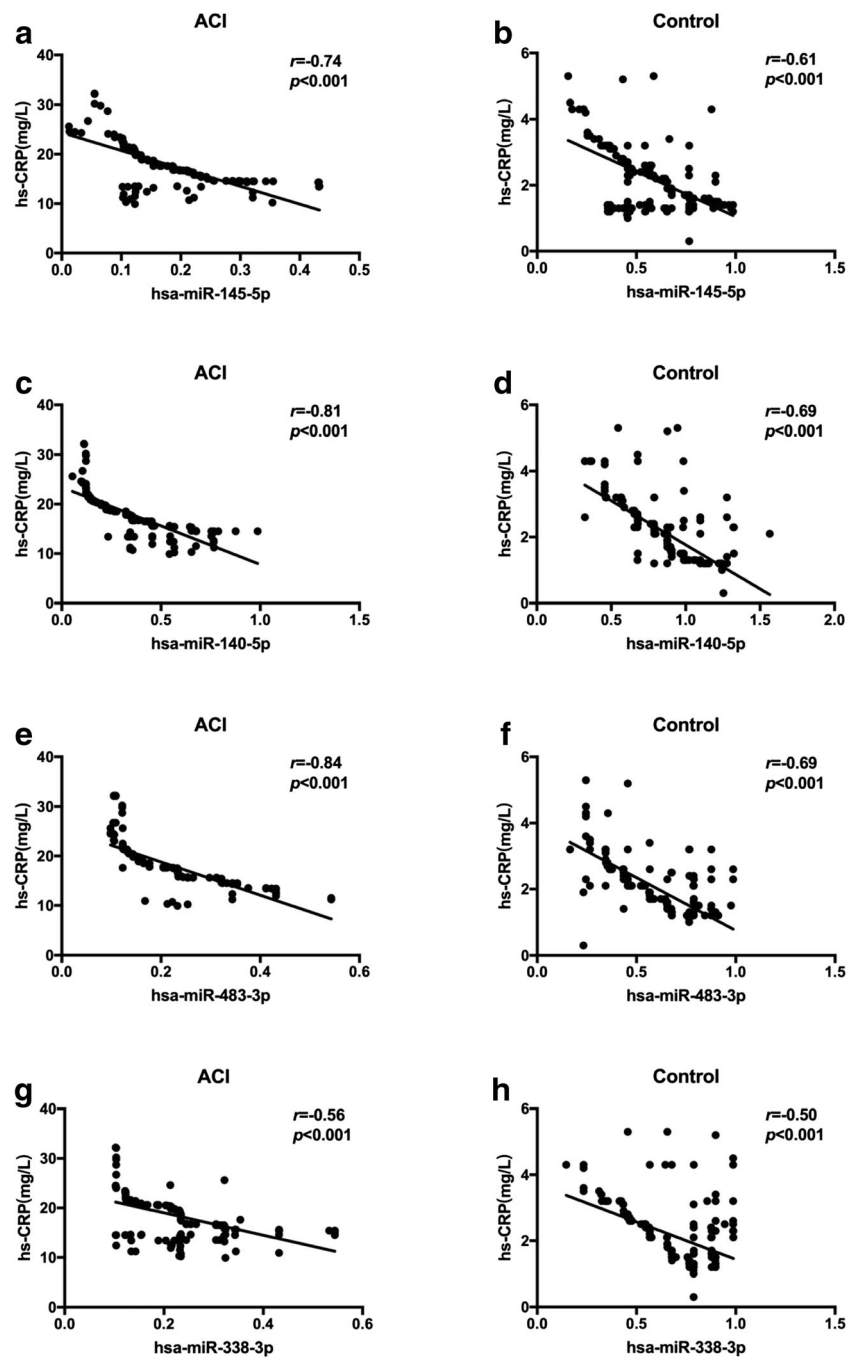

HUVECs, and miRNAs were quantified at 48 h post-transfection. As shown in Fig. 6, overexpression of MALAT1 significantly inhibited the expression of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p ($p < 0.05$). On the contrary, knockdown of MALAT1 markedly enhanced the expression of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p ($p < 0.001$). These results demonstrated that MALAT1 negatively regulated the expression of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p.

miRNAs Inhibit CRP mRNA Transcription in HUVECs

In order to detect the effect of miRNAs including hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p on CRP mRNA level, specific miRNA inhibitors and mimic miRNAs of the four miRNAs were added into HUVECs and CRP mRNA expression was quantified by RT-PCR. As shown in Fig. 7, compared with blank control, HUVECs with inhibitors addition expressed more CRP mRNA while transcription of CRP mRNA was significantly suppressed in HUVECs with miRNAs mimic.
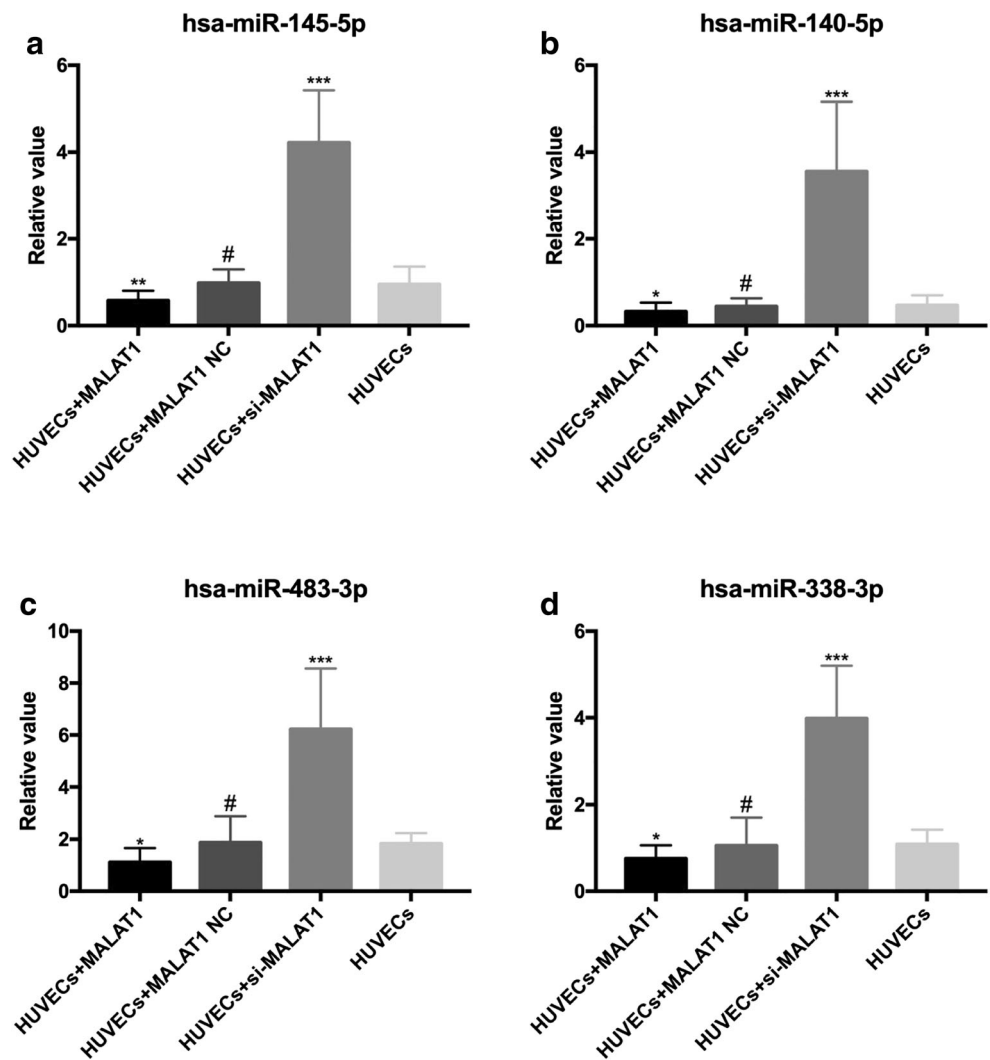
miRNA Negatively Regulated hs-CRP Expression

There was a negative correlation between miRNA and hs-CRP expression level in plasma (Fig. 5), which also suggested miRNA may be involved in the regulation of hs-CRP expression. Specific inhibitors and mimic of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p were transfected into HUVECs, and cells without transfection were used as blank control. At 48 h post-transfection, all the cells were harvested to extract protein for CRP level detection. From Fig. 8, it could easily find that inhibition of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p dramatically increased CRP level while overexpression of those miRNAs suppressed CRP expression, indicating hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p were capable of negatively regulating CRP level.

Discussion

Atherosclerosis is a chronic inflammatory process, and it plays an important role in the development of ACI (Deguchi et al. 2006). CRP, synthesized in the liver, is one of the acute phase proteins and a cyclic interpolymer composed of 5 identical subunits non-covalently bonded (Hind et al. 1985). CRP is rarely expressed in plasma, but its circulating concentration is extremely increased in response to the stimulation of tissue damage, infection, and inflammation. CRP has a pro-inflammatory effect on vascular cells and may play a causal role in the pathogenesis of coronary artery disease. Previous studies have shown that individuals with an increased amount of CRP have a greater risk of stroke and myocardial infarction (Ridker et al. 1998). Besides, CRP binds with high affinity to phosphatidylcholine expressed on the surface of dead or dying cells and some kinds of bacteria, thereby activating complement and promoting phagocytosis of macrophages (Pepys and Hirschfield 2003).

Fig. 7 miRNAs inhibit CRP mRNA transcription in HUVECs. **a** Comparison of CRP mRNA level in hsa-miR-145-5p mimic, hsa-miR-145-5p inhibitor, and the control groups in HUVECs. **b** Comparison of CRP mRNA level in hsa-miR-140-5p mimic, hsa-miR-140-5p inhibitor, and the control groups in HUVECs. **c** Comparison of CRP mRNA level in hsa-miR-483-3p mimic, hsa-miR-483-3p inhibitor, and the control groups in HUVECs. **d** Comparison of CRP mRNA level in hsa-miR-338-3p mimic, hsa-miR-338-3p inhibitor, and the control groups in HUVECs. $*p < 0.05$; $**p < 0.01$, compared with control
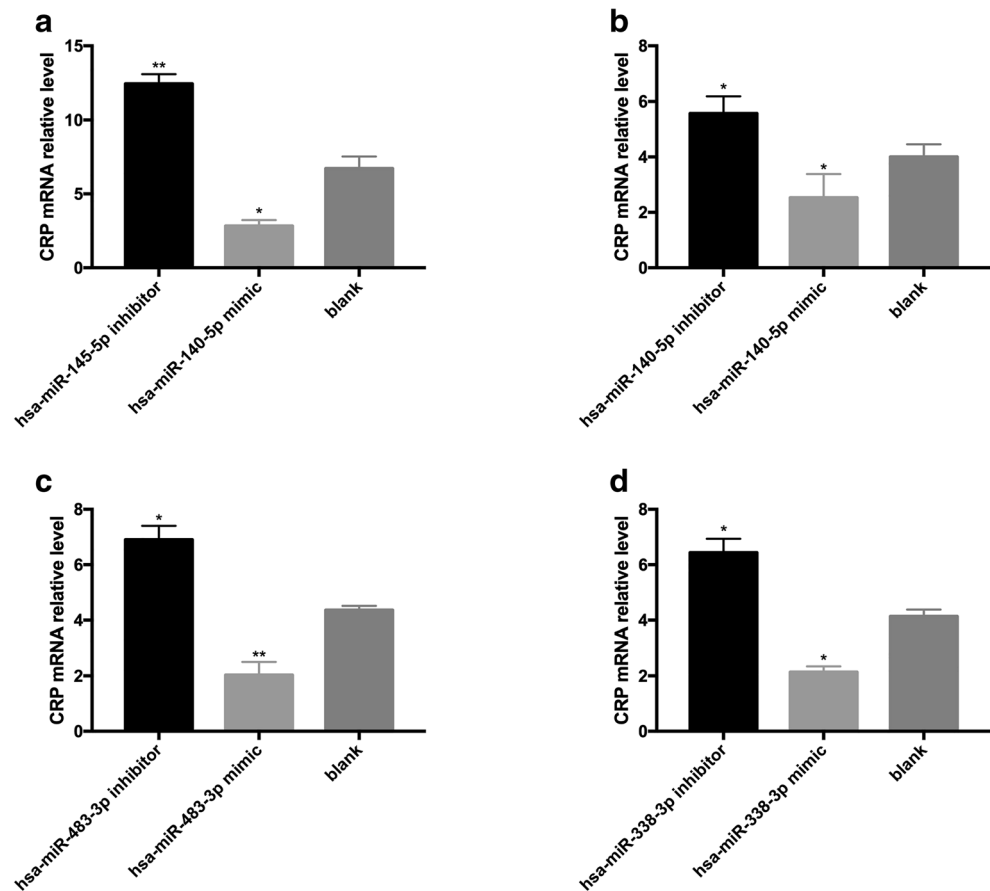

In this study, CRP levels in the plasma of ACI patients $((18.33 \pm 3.71)$ mg/L) were significantly higher than those in the control group $((2.24 \pm 0.83)$ mg/L). The plasma detected in this study was collected within 6 h after the onset of ACI, and it generally takes more than 6 h to synthesize CRP in the liver. Therefore, results indicated that CRP in patients had a high level in the period before the onset of cerebral infarction, and CRP played a crucial role in the occurrence of ACI.

A week after the disease is the progressive stage of the disease, at which time the tissue damage is the most serious. During this period, the infiltration of mononuclear cells in the inflammatory reaction accompanied by cerebral infarction occurs, and the amount of inflammatory mediators including CRP synthesized and secreted by the liver increase significantly.

MALAT1 is a highly enriched and conserved LncRNAs with a length of 8000 nt and located on chromosome 11q13 (Tripathi et al. 2010). MALAT has been shown to be involved in the pathophysiological processes of various cardiovascular diseases (Zhang et al. 2016a; Han et al. 2018). Han et al. found
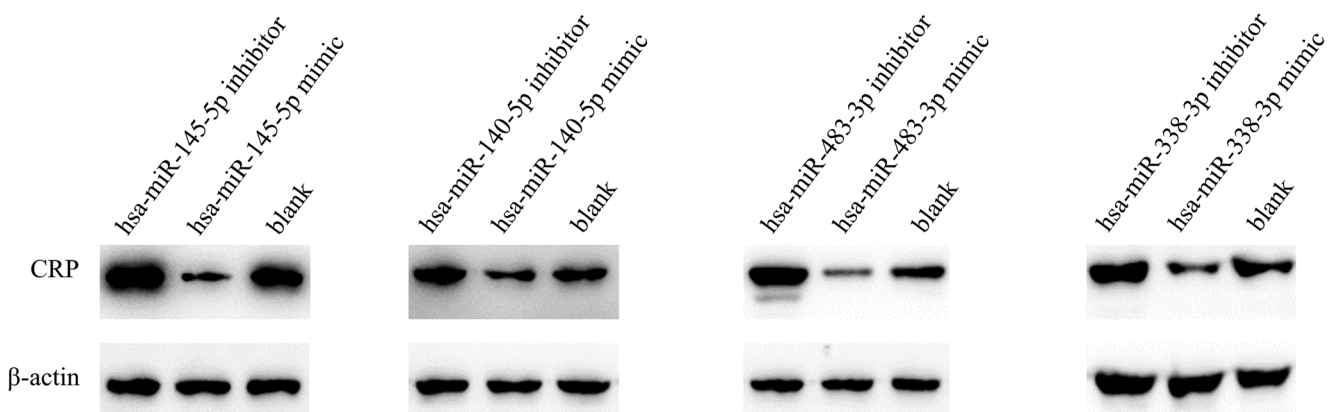
Fig. 8 Hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, hsa-miR-338-3p downregulated CRP protein expression

that MALAT1 is increased in macrophages of diabetic atherosclerotic rats, suggesting that MALAT1 is closely related to atherosclerosis (Han et al. 2018). In order to investigate whether LncRNA MALAT1 is associated with the development of ACI, we measured the plasma levels of MALAT1 in ACI patients and controls. The results showed that the level of MALAT1 in plasma of ACI patients was significantly higher than that of the control group ($p < 0.001$) (Fig. 3), which was consistent with the previous study (Zhang et al. 2016b). However, the specific mechanism is still not clear at present. To answer the question, we analyzed the potential mechanism of MALAT1 in ACI development and the interaction among MALAT1, hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, hsa-miR-338-3p, and CRP.

The plasma levels of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in ACI patients were significantly lower than those in the control groups ($p < 0.001$) (Fig. 2). Further studies found that the plasma levels of MALAT1 were negatively correlated with the level of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p in both ACI patients and the control groups (Fig. 4). These results suggest that MALAT1 may negatively regulate the expression of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p. But, this hypothesis needs to be confirmed in in vitro studies. Surprisingly, in ACI patients, the suppression of miR-140 wears off at higher levels of MALAT1. We hypothesize that the complex in vivo condition may affect the regulation of the expression of hsa-miR-140-5p, which may lead to the inconsistent results in vitro and in vivo.

In fact, the MALAT1-microRNA-gene regulatory network has been identified to be involved in the occurrence and development of a variety of diseases. Downregulation of MALAT1 dramatically attenuated neuronal cell death through suppressing beclin1-dependent autophagy by regulating miR30a in cerebral ischemic stroke (Guo et al. 2017). By using TargetScan, we predicted that hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p can target to the 3′ non-coding region (UTR) of CRP gene. Combined with the results that the plasma level of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p were negatively correlated with the level of CRP in ACI patients and the control groups (Fig. 5), we hypothesized that hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p were capable of downregulating the CRP expression.

In order to further investigate the MALAT1-miRNAs-CRP regulatory network, we overexpressed MALAT1 in HUVECs and quantified miRNA levels. Transfection of MALAT1 significantly decreased the level of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p, which supported the idea that MALAT1 downregulated hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p

expressions (Fig. 6). Meanwhile, hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p suppressed CRP expression because overexpression of these microRNAs also decreased the level of CRP protein (Fig. 7).

Conclusion

Our experiments revealed that LncRNA MALAT1 downregulated the expression of hsa-miR-145-5p, hsa-miR-140-5p, hsa-miR-483-3p, and hsa-miR-338-3p, while the downregulation of these microRNAs, in turn, may lead to the upregulation of CRP expression. Therefore, the LncRNA MALAT1-miRNAs-CRP regulatory network is promising as a therapeutic target for ACI diagnosis and treatment.

Compliance with Ethical Standards

The experimental scheme was approved by the Ethics Committee of Shanghai East Hospital, and all patients and healthy individuals signed informed consent forms.

Conflict of Interest The authors declare that they have no conflict of interest.

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