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LncRNA HULC alleviates HUVEC inflammation and improves angiogenesis after myocardial infarction through down-regulating miR-29b

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Abstract. – OBJECTIVE: The aim of this study was to investigate the long non-coding RNA (lncRNA) HULC in promoting angiogenesis after myocardial infarction (MI) and to further investigate its possible mechanism.

MATERIALS AND METHODS: Twenty-four male Sprague Dawley (SD) rats were randomly divided into two groups, namely, operation group and control group. The rats in the operation group were induced by ligation of the left anterior descending coronary artery, while those in control group received the same surgery without ligating the blood vessels. Seven days after the operation, the myocardial tissues of rats were collected to detect HULC expression by quantitative real-time polymerase chain reaction (RT-PCR). At the same time, the expression of HULC in primary myocardial cells and cardiac microvascular endothelial cells were induced by hypoxia. A hypoxia model was constructed in HUVEC cells, and the effects of HULC were explored by RT-PCR, Western blot Technology (WB), Cell Counting Kit-8 (CCK8) assay, EdU staining, Tube-like structure formation experiments. Thereafter, HULC downstream miRNAs were verified by Luciferase, pull-down, and RNA IP experiments. Similarly, the effects of miR-29b on HUVEC were verified by RT-PCR, WB, CCK8 assay, EdU staining, and tube-like structure formation experiments, respectively.

RESULTS: RT-PCR detection results showed that the expression of HULC in myocardial tissues was down-regulated after MI, and the expression of HULC in cardiac microvascular endothelial cells was decreased under hypoxia-induced inflammation. In addition, the overexpression of HULC in HUVEC cells could inhibit the expressions of inflammatory factors (IL-1, IL-6 and IL-8) and promote angiogenesis (increased cell viability, increased tube-like structure formation, and increased cell proliferation). Through Dual-Luciferase reporter gene experiments, it was found that HULC could directly target miR-29b. At the same time, miR-29 overexpression significantly reversed the effects of HULC on cell viability, pro-inflammatory cytokines, and angiogenesis.

CONCLUSIONS: LncRNA HULC protects HUVEC cells from hypoxia-induced inflammation damage by interacting with miR-29b and inhibiting its expression, and it can also promote angiogenesis.

Key Words:
LncRNA HULC, MiR-29b, Angiogenesis, Inflammation, Myocardial infarction.

Introduction

Therapeutic angiogenesis, a therapeutic method that promotes angiogenesis in the ischemic region of the heart, is a new strategy to increase blood supply to the myocardium of patients with ischemic heart disease[1]. This treatment provides a new direction for patients who are not suitable for standard revascularization techniques. Therefore, therapeutic angiogenesis in acute myocardial infarction (AMI) has significant clinical potential[2].

Most human genome sequences (70-90%) are transcribed into RNA molecules, which can be encoded into protein products after translation and processing, and then play a role in various physiological metabolisms. However, there are other RNAs in the body that do not participate in encoding protein products, which are known as non-coding RNA (ncRNA). They are in the form of RNA itself at various levels (epigenetic regulation, transcription regulation and post-transcriptional regulation, etc.), regulate the expression level of genes, and occupy the largest part of the human genome. Some of these ncRNA sequences are highly conserved, exhibit a regulatory and tissue-specific expression, and play a key role in cells. Their abnormal expression or mutation can

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cause epigenetic perspectives[3]. Long non-coding RNAs (lncRNAs) are a class of endogenous RNAs longer than 200 nucleotides in length and have a strong regulatory effect on gene expression or post-transcriptional processing. The abnormal expression of lncRNAs can affect the occurrence, development and regulation of various ischemic diseases including myocardial infarction (MI)[4,5]. Importantly, lncRNA-H19 can inhibit cardiomyocyte apoptosis by binding to miR-29b[6], and lncRNA Gm2691 inhibits inflammation and reduces myocardial cell apoptosis[7]. With the deepening of research, lncRNA has been reported to be involved in tissue repair after ischemia, and lncRNA MIAT can promote angiogenesis after MI through the PI3K/AKT pathway[8].

LncRNA HULC (highly up-regulated in liver cancer) is the first non-coding transcript proved to be strongly overexpressed in human hepatocellular carcinoma[9]. HULC gene is located on the 3rd band of the 24 regions of the short arm of chromosome 6, and it is evolutionarily conserved among primates. The transcription of HULC gene can generate a spliced, polyadenylated, non-coding RNA of approximately 500 nt in length, which is localized in the cytoplasm and has been reported to be involved in ribosomal function. HULC expression is regulated by the transcription factor CREB [cyclic adenosine monophosphate (CAMP) response element binding protein][10,11]. Increased HULC expression in cells can induce cell proliferation and tumor growth, and lead to the down-regulation of tumor suppressor p18[12]. Moreover, HULC is involved in the regulation of atherosclerosis through DNA methylation and binding to miR-9[13]. However, the expression, role, and regulatory mechanism of HULC in MI are largely unknown.

Materials and Methods

Animal Experiment

This investigation was approved by the Animal Ethics Committee of Wenzhou Central Hospital Animal Center. 8-week-old Specific Pathogen Free (SPF)-grade Sprague Dawley (SD) rats (Huafukang, Beijing, China) were used to construct rat models of MI, and the rats were given a moderate degree of anesthesia based on body weight with freshly prepared sodium valproate (Camilo Biological, Nanjing, China). The rat's abdomen, limbs and teeth were fixed, and then the limbs were monitored by ECG (Olympus, Tokyo, Japan). Then, their hair was cut in the middle of the neck and left chest of the rats, the skin was cut, and the subcutaneous fat and soft tissues were separated with a hemostat. After trachea was observed, it was cut open, connected to a ventilator and fixed. After the chest was opened, the pericardium was exposed, the pericardium was cut and then the left atrial appendage was separated. In the operation group, 6-0 sutures (HaoXin, Quanzhou, China) were used to hook and ligate the appropriate heart tissues. In the control group, the operation was the same as before without ligation. If it was observed that the heart ligation site became pale, and the weakening of the jumping force was accompanied by the evolution of the electrocardiogram, the rats suffered from MI. After making sure there was no blood in the chest cavity, the intercostal muscles and ribs were sutured. After the chest suture was determined, the trachea cannula was pulled out, and the open trachea was sutured after the rats resumed spontaneous breathing. Penicillin should be applied for 3 consecutive days after the operation to prevent infection.

Cell Culture

Primary myocardial cells (Cell Culture Center, Shanghai, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Life Technology, Wuhan, China) with 15% fetal bovine serum (FBS) (Life Technology, Wuhan, China), 100 IU/mL penicillin and 100 μg/mL streptomycin, and cardiac microvascular endothelial cells (Cell Culture Center, Shanghai, China) were cultured in endothelial cell culture solution containing 5% FBS and 1% Endothelial Cell Growth Supplement (ECGS, Life Technology, Wuhan) added with 100 IU/mL Penicillin and 100 μg/mL streptomycin. In addition, 293T and HUVEC (Cell Culture Center, Shanghai, China) were cultured in DMEM containing with 10% FBS,100 IU/mL penicillin and 100 μg/mL streptomycin. The above cells were all cultured in an incubator containing 5% CO_2 at 37°C, and the cell confluence reached 70-80% (about 2-3 days). 293T cells, HUVEC cells and cardiac microvascular endothelial cells were digested with 0.25% trypsin (1: 2), and 3rd to 4th generation cells were used for experiments.

Quantitative Real-Time Polymerase Chain Reaction (RT-PCR)

In strict accordance with the instructions of the SuperReal PreMix Plus (SYBR Green, Thermo

Fisher Scientific, Waltham, MA, USA) kit, the reagents were amplified at room temperature and placed on ice. Then, the complementary deoxyribose nucleic acid (cDNA) and reagents were fully mixed and placed in the PCR instrument (Olympus, Tokyo, Japan) under the following reaction conditions: (95°C for 15 minutes, 95°C for 10 seconds and 60°C for 30 seconds) ´ 45 cycles. For each pair of primers, three experiments were repeated in each template, and the Ct values obtained were averaged. The Ct value of each sample was obtained using ABI primer 7000 SDS software (Applied Biosystems, Foster City, CA, USA). The average Ct of each target gene minus the average Ct of the internal reference gene (GAPDH) of the corresponding template to obtain ΔCt, ΔΔCt = experimental group $^{\Delta}$Ct - control group ΔCt, and the final calculation formula Fold changes = $2^{-\Delta\Delta Ct}$. Primers used were shown in Table I.

Western Blot Technology (WB)

The total protein of each group of cells was extracted with radioimmunoprecipitation assay (RIPA) lysate (Camilo Biological, Nanjing, China), and its concentration was detected by bicinchoninic acid (BCA) kit (Camilo Biological, Nanjing, China). Then, the protein was separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis, transferred to a polyvinylidene difluoride (PVDF, Thermo Fisher Scientific, Waltham, MA, USA) membrane by semi-dry transfer method, and blocked with 5% skim milk powder at room temperature for 2 h. Then, primary antibodies against GAPDH (1:1000, Abcam, Cambridge, MA, USA), IL-1 (1:1000 Abcam, Cambridge, MA, USA), IL-6 (1:1000, Abcam, Cambridge, MA, USA), IL-8 (1:1000, Abcam, Cambridge, MA, USA) were added for incubation at 4°C overnight. After the PVDF membrane was washed for three times with Tris-Buffered Saline and Tween-20 (TBST) solution, enhanced chemiluminescence (ECL) chemiluminescence solution (Thermo Fisher Scientific, Waltham, MA, USA) was added and quickly placed in a gel imaging system to collect and analyze images.

Cell Counting Kit-8 (CCK8) Assay

Operated strictly in accordance with the instructions of CCK-8 kit (JianCheng, Nanjing, China), cells were inoculated in 96-well plates, with 100 μL per well. These cells were assigned into control group, experimental group and blank control group, with at least 5-6 parallel wells each. 96 wells were filled with phosphate-buffered saline (PBS) to avoid the liquid evaporates, and the culture plate was pre-incubated in the incubator for 16 h. According to the experimental grouping, the normal or sugar-free medium was replaced, and the corresponding hypoxia/reoxygenation treatment was performed. When the processing time went by, 10 μL of CCK-8 solution was added to each well for 1.5 h of incubation in the incubator. Finally, the absorbance of each well at 450 nm was measured with a microplate reader, and cell proliferation was calculated based on the following formula: cell proliferation (OD control group-OD experimental group)/(OD control group-OD blank control) × 100%.

5-Ethynyl-2′- Deoxyuridine (EdU) Staining

Cells were digested with 200 μL of 0.25% trypsin, and the wells were thoroughly washed with pre-chilled phosphate-buffered saline (PBS) until no evident adherent cells were seen under the microscope. Then, the cells were collected into 15 mL centrifuge tubes and counted in each well. Then, the unbound EdU molecules (JianCheng, Nanjing, China) were removed by centrifugation, and the cells were resuspended by adding 1 mL of 40 g/L paraformaldehyde and

Table I. Real time PCR primers.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
IL-1	TTGAGTCTGCCCAGTTCC	TTTCTGCTTGAGAGGTGCT
IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
IL-8	ACCACACTGCGCCAACACAGAAAT	TCCAGACAGAGCTCTCTTCCATCAGA
Lnc HULC	ATCTGCAAGCCAGGAAGAGTC	CTTGCTTGATGCTTTGGTCTGT
miR-29b	AGGCTAGCACCATTTGAAATC	GAGAGGAGAGGAAGAGGGAA
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-PCR, quantitative reverse-transcription polymerase chain reaction.

fixed at room temperature for 30 min. Following washing with PBS, the cells were added 200 μL of penetrant (0.5% TritionX-100, Camilo Biological, Nanjing, China) for permeation, followed by incubation for 10 minutes in a shaker. Next, the cells were washed with PBS, added with 200 μL of 1 × Apollo staining reaction solution, and incubated for 30 minutes in the dark. Subsequently, the cells were washed with PBS, added with 200 μL 4',6-diamidino-2-phenylindole (DAPI) reaction solution in each tube, and incubated for 30 minutes in the dark. After washing with PBS again, the cell concentration was adjusted to (1.0-2.0) × 10^9 / L, with a volume of 200 μL, and the samples were kept from light. Finally, the films were allowed to dry at room temperature, added with an anti-fluorescent quencher (Sanggon, Shanghai, China), and observed under a fluorescence microscope.

Pull-Down

A biotinylated DNA probe complementary to HULC was synthesized and dissolved in binding and washing buffers. Later, the solution was incubated with Dynabeads M-280 avidin (Fitgene, Guangzhou, China) at room temperature for 10 minutes. Next, the HUVEC cell lysate was incubated with the probe-coated magnetic beads for a period of time to elute the RNA complex binding to the magnetic beads and determine the concentration and quality of the RNA. Thereafter, the purified RNA was analyzed by RT-PCR.

RNA Immunoprecipitation

HUVEC cell culture medium was added with protease inhibitor and RNA lysis buffer of EZ-magna RIP RNA binding protein immunoprecipitation kit (Camilo Biological, Nanjing, China). RNA immunoprecipitation was performed according to the instructions. Next, anti-Argonaute2 (Agog) antibodies and normal mouse IgG-bound magnetic beads were incubated in RIP immunoprecipitation buffer and cell lysate was added. Later, the samples were treated with proteinase K buffer, and the precipitated RNA was isolated. At last, the concentration and quality of RNA were measured, and the purified RNA was detected by RT-PCR.

Luciferase Gene Report Experiment

293T cells were separately transfected with pRL-HULC-WT, pRL-HULC-Mut, miR-29b mimics and miR-29b NC (Thermo Fisher Scientific, Waltham, MA, USA). A total of 40 ng of control plasmids and 800 ng of recombinant plasmids were transfected in each group. The final concentration of mimics and NC was 20 nmol / L. Three replicates were set in each group. After 48 h, the cells were lysed and detected using a Dual-Luciferase detection kit (Bio-Rad, Hercules, CA, USA). The chemiluminescence meter was used to measure the data of each well and subtract the basal signal of the blank wells. Lastly, the firefly fluorescence signal from the pGL3 plasmid was used as a control, and the relative fluorescence activity was determined by dividing the fluorescence signals from the recombinant plasmids pRL-HULC-WT and pRL-HULC-Mut by the firefly fluorescence signals.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was used for statistical analysis of experimental data. All data were expressed as mean ± SD (standard deviation). Three groups of data were compared using ANOVA, and the comparison between each two groups was tested by q test. Two independent samples were compared using *t*-test. $p<0.05$ was considered statistically significant.

Results

Down-Regulation of HULC Expression In Myocardial Tissue of MI Rats

In this experiment, the relative expression levels of proinflammatory cytokines IL-1, IL-6, and IL-8 in tissues were detected by RT-PCR. The results showed that the expressions of IL-1, IL-6 and IL-8 in the myocardial tissues of MI rats were dramatically higher than those in Sham group (Figure 1A-1C), and expression of HULC in the myocardial tissues of MI rats was significantly reduced compared with that in normal tissues (Figure 1D). These data suggest that lncRNA HULC may play a key role in the development of MI. At the same time, cell hypoxia models were constructed in primary cardiomyocytes and cardiac microvascular endothelial cells, and it was found that HULC had no difference between the normoxic and hypoxic groups of cardiomyocytes (Figure 1E). However, the expression of HULC in cardiac microvascular endothelial cells in the hypoxic group was significantly lower than that in the Control group (Figure 1F).

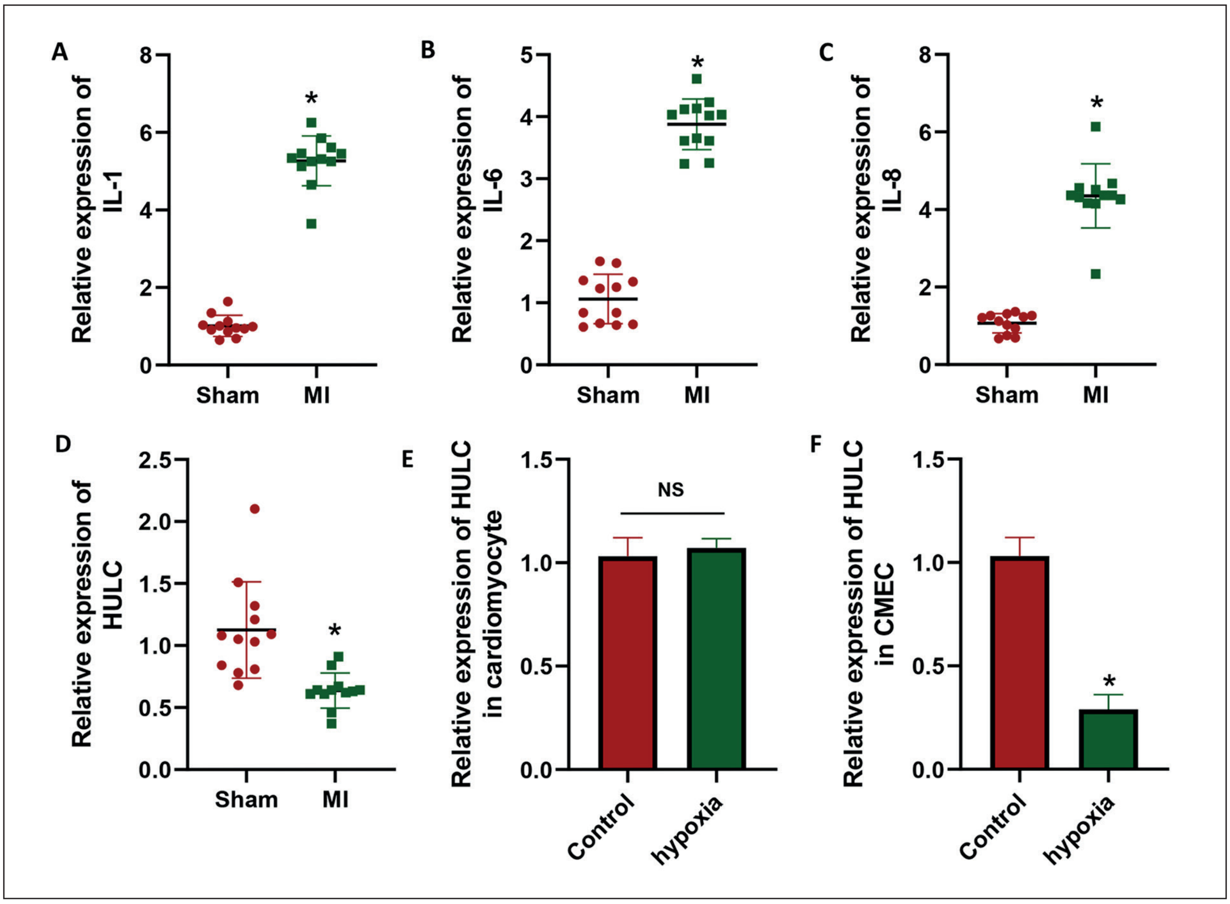

Figure 1. Down-regulation of HULC expression in the myocardial tissues of MI rats. **A-C,** The expression levels of inflammatory cytokines (IL-1, IL-6 and IL-8) in the heart tissues ("*" $p<0.05$ *vs.* Sham group). **D,** The expression levels of HULC in the heart tissues ("*" $p<0.05$ *vs.* Sham group). **E,** The expression levels of HULC cardiomyocytes. **F,** The expression levels of HULC in the rat cardiac microvascular endothelial cells ("*" $p<0.05$ *vs.* Sham group).

HULC Could Reduce the Inflammatory Damage and Promote the Proliferation of HUVEC Cells Induced by Hypoxia

To verify whether HULC plays a role in cardiac microvascular endothelial cells, HUVEC cells were transfected with pc-HULC and pcDNA3.1 to change the expression of lncRNA HULC. The results revealed that HULC expression was significantly down-regulated in the hypoxic group compared with that in the Control group, while HULC expression was significantly up-regulated in pc-HULC transfected cells (Figure 2A). Next, changes in cell viability, pro-inflammatory cytokines, and angiogenesis after plasmid transfection were examined. It was discovered that under hypoxia, HULC significantly increased the viability of HUVEC cells (Figure 2B) and inhibited the mRNA and protein expression levels of IL-1, IL-6 and IL-8 (Figure 2C and D). At the same time, it promoted the proliferation of HUVEC cells. EdU staining showed that HUVEC-positive cells were dramatically increased after HULC overexpression (Figure 2E). Tube-like structure formation experiments found that after 16 h of culture, compared with the pcDNA3.1 group, the cumulative tube length was significantly increased in HULC overexpression group (Figure 2F). The above results indicate that HULC can reduce the inflammatory damage and promote the proliferation of HUVEC cells induced by hypoxia.

HULC can be Directly Targeted to MiR-29b

As one of the important microRNAs, miR-29b has been reported to play a key role in different diseases. In the current study, it was speculated that miR-29b may be involved in the regulation of the effects of HULC on the hypoxia-induced

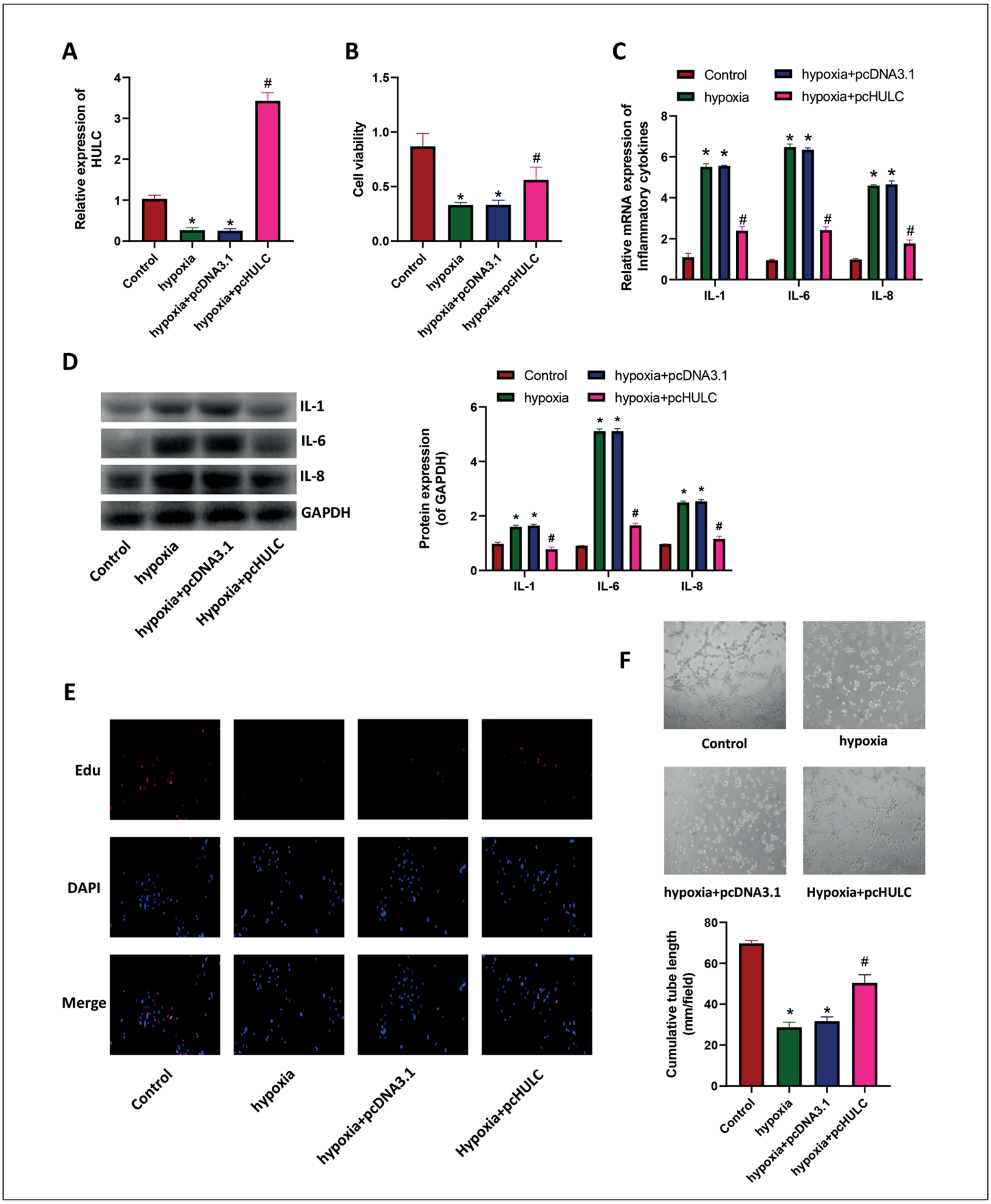

Figure 2. HULC could reduce the inflammatory damage and promote the proliferation of HUVEC cells induced by hypoxia. **A,** The expression levels of HULC in the HUVEC cells ("*" $p<0.05$ *vs.* Control group, "#" $p<0.05$ *vs.* hypoxia+pcDNA3.1 group). **B,** CCK8 assay showed the viability of HUVEC cells ("*" $p<0.05$ *vs.* Control group, "#" $p<0.05$ *vs.* hypoxia+pcDNA3.1 group). **C,** The mRNA expression levels of inflammatory cytokines (IL-1, IL-6 and IL-8) in HUVEC cells ("*" $p<0.05$ *vs.* Control group, "#" $p<0.05$ *vs.* hypoxia+pcDNA3.1 group). **D,** Western blot bands and gray value analysis of inflammatory cytokines (IL-1, IL-6 and IL-8) in HUVEC cells ("*" $p<0.05$ *vs.* Control group, "#" $p<0.05$ *vs.* hypoxia+pcDNA3.1 group). **E,** EdU staining. (magnification: 400×) **F,** Representative images of capillary-like structures and quantitative analysis of the total tube length (magnification: 40×) ("*" $p<0.05$ *vs.* Control group, "#" $p<0.05$ *vs.* hypoxia+pcDNA3.1 group).

HUVEC cell inflammation and proliferation. First, the relative expression of miR-29b was examined in cells after pc-HULC transfection, and it was revealed that compared with pcDNA3.1, HULC overexpression significantly reduced the expression level of miR-29b (Figure 3A). Next, to explore the relationship between HULC and miR-29b, Targetscan was used to predict the possible interaction sites between the two. Then, the 3'-UTR binding sequence of the HULC gene was mutated and cloned into a fluorescent vector, which was then applied to co-transfect the cells with miR-29b. Moreover, the experimental analysis of the Dual-Luciferase reporter gene manifested that miR-29b could inhibit the Luciferase activity of the wild-type HULC transfection group. However, there were no significant changes in the mutant HULC group (Figure 3B). Later, whether miR-29b can interact with HULC was investigated using the biotin-avidin Pull-down system. HUVEC cells were transfected with the biotin-labeled wild-type miR-29b gene. Besides, RT-PCR analysis showed that after the avidin adsorption, the HULC concentration in the system was increased, while the mutant miR-29b could not change the amount of HULC, suggesting that HULC has a direct interaction with miR-29b (Figure 3C). As mature miRNAs can form RNA silencing complexes (RISC) with the Argonaute (Ago) protein family, which can degrade target genes or inhibit transcription, anti-Ago2 antibodies were utilized for RNA immunoprecipitation experiments. It was demonstrated that in HUVEC cells expressing Ago2, HULC and miR-29b expression levels were significantly up-regulated (Figure 3D), revealing that HULC and miR-29b could be bound by Ago2 simultaneously. The above results indicate that HULC functions in HUVEC cells by binding to miR-29b, and there may be a mutual inhibitory effect between HULC and miR-29b.

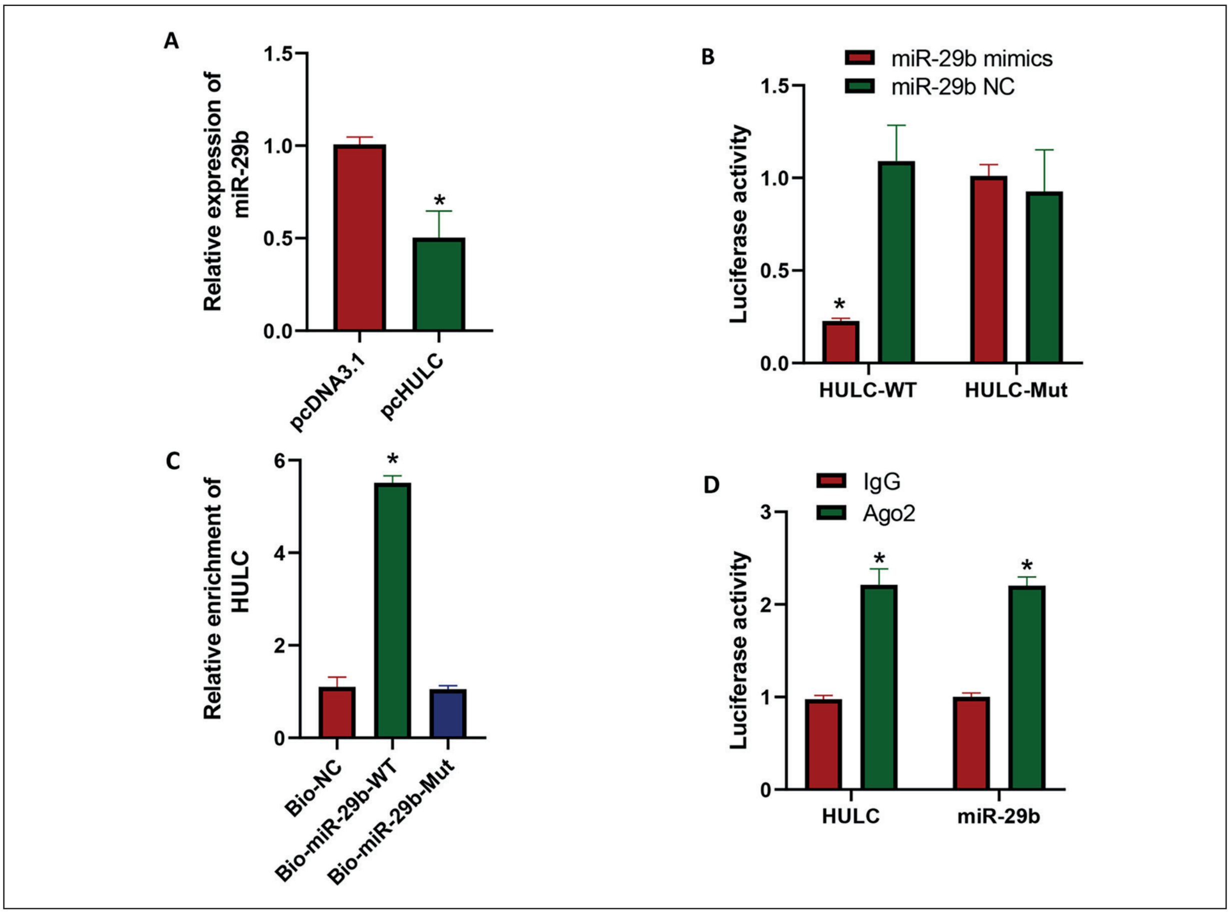

Figure 3. HULC could be directly targeted to miR-29b. **A,** The expression levels of miR-29b in HUVEC cells ("*" $p<0.05$ *vs.* pcDNA3.1 group). **B,** Dual-Luciferase gene assay ("*" $p<0.05$ *vs.* HULC-WT+miR-29b NC group). **C,** RT-PCR was used to determine the enrichment of HULC in the samples ("*" $p<0.05$ *vs.* Bio-NC group). **D,** The interaction between HULC and miR-29b was evaluated by anti-Ago2 antibody RNA immunoprecipitation method ("*" $p<0.05$ *vs.* IgG group).

HULC Reduced Hypoxia-Induced Inflammatory Injury and Promotes Proliferation of HUVEC Cells by Regulating the Expression of MiR-29b

MiR-29b mimics and miR-29b NC were transfected into HUVEC cells. RT-PCR detection showed that miR-29b expression in cells transfected with miR-29b mimics was significantly up-regulated in comparison with that in miR-29b NC group (Figure 4A), indicating that the successful transfection can be used for functional research experiments. Subsequent experimental results verified that in hypoxia-treated HUVEC cells, the overexpression of miR-29b markedly reversed the effect of HULC on the cell viability, proinflammatory cytokines and proliferation, manifested as decreased cell viability (Figure 4B), increased inflammatory factor damage (Figure 4C and 4D), and inhibition of proliferation (Figure 4E and 4F). These data indicate that miR-29b overexpression suppresses the protective effect of HULL on HUVEC cells induced by hypoxia.

Discussion

The main findings of this study included: (1) LncRNA HULC expression was down-regulated in the myocardial tissues of MI rats, and HULC expression was also down-regulated in cardiac microvascular endothelial cells under hypoxia. (2) HULC reduced hypoxia-induced inflammatory damage to HUVEC cells and promoted proliferation. (3) HULC down-regulated the expression of miR-29b in HUVEC cells. (4) MiR-29b inhibited the protective effect of HULC on HUVEC cells injured by hypoxia.

AMI has been the leading cause of morbidity and death worldwide for a long time. Therapeutic angiogenesis is a treatment method that can facilitate the formation of new blood vessels to improve myocardial ischemia and hypoxia by transporting some cytokines that promote neovascularization to ischemic cardiovascular endothelial cells in some way[14]. Now this kind of treatment is considered as a new treatment direction to improve the cardiac blood supply of patients with MI, and also a new treatment option for heart patients who cannot receive bypass surgery or PCI[15]. The therapeutic angiogenesis in AMI has significant clinical potential. Over the past 20 years, therapeutic angiogenesis has been extensively validated in animal models of chronic myocardial ischemia, as well as in several human clinical trials[16]. VEGF can promote the formation of collateral blood vessels, and improve myocardial perfusion and cardiac function[17]. However, its clinical benefits have not been confirmed by large-sample, multi-center, and placebo-controlled experimental results mainly due to unclear mechanisms, technical barriers to treatment, and clinical design constraints[18]. As VEGF has a negative effect on the ischemic heart, tissue damage after MI is aggravated by inducing the breakdown of endothelial cells (EC), thereby aggravating vascular leakage, inflammation and hypoxia[19]. Due to time constraints, the downstream target genes of miR-29b have not been researched in our study. However, Li et al[20] showed that overexpression of miR-29b suppressed the angiogenesis of cervical cancer cells *in vitro* through targeting STAT3 signal pathway. In addition, Tang et al[21] found that overexpression of miR-29b-3p remarkably inhibited the function of RMECs in cell proliferation and angiogenesis by regulating VEGFA and PDGFB. Similarly, it was found in this study that miR-29b may target these genes for its role in angiogenesis.

LncRNA HULC is a non-coding RNA, and it has been shown to play a role in vascular endothelial cell injury. Besides, it has been revealed that lncRNA MALAT1[22], TUG1[23] and MEG3[24] exert functional effects on endothelial cells and angiogenesis. Studies have demonstrated that lncRNA H19[6] inhibits glioma-induced endothelial cell proliferation, migration and angiogenesis by upregulating miR-29a, and regulates the occurrence of glioma by regulating the biological behavior of glioma vascular endothelial cells. Overall, HULC's emerging role is important. Therefore, understanding the molecular mechanism and pathological effects is crucial for realizing the therapeutic potential, improving the prognosis and providing better therapeutic effect for patients.

There are also some limitations in this report. The oxygen-sensitive transcription factor HIF-1α is a known transcription factor for several inflammatory cytokines including IL-1β and IL-6, and it significantly regulates the production of ILs both at the transcript and protein levels. In this study, vascular expressions such as HIF-1 expression leading to overproduction of ILs were not considered. In addition, proangiogenic effects of miR-29b should be verified *in vivo* using animal models in future investigations.

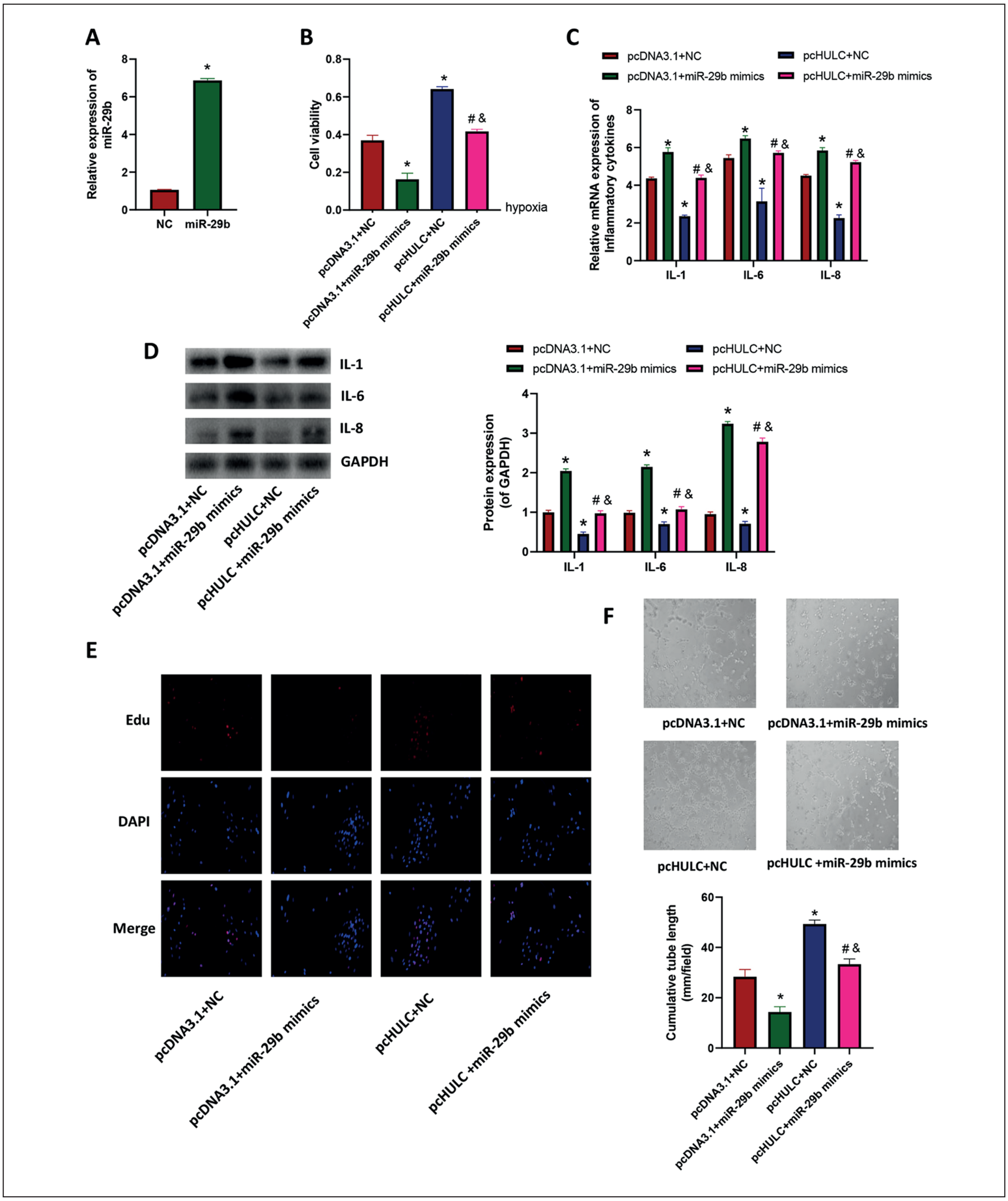

Figure 4. HULC reduces hypoxia-induced inflammatory injury and promotes proliferation of HUVEC cells by regulating the expression of miR-29b. **A,** The expression levels of miR-29b in HUVEC cells ("*" $p<0.05$ *vs.* NC group). **B,** CCK8 assay shows the cell viability of HUVEC cells ("*" $p<0.05$ *vs.* pcDNA3.1+NC group, "#" $p<0.05$ *vs.* pcDNA3.1+miR-29b mimics group, "&" $p<0.05$ *vs.* pcHULC+NC). **C,** The mRNA expression levels of inflammatory cytokines (IL-1, IL-6 and IL-8) in HUVEC cells ("*" $p<0.05$ *vs.* pcDNA3.1+NC group, "#" $p<0.05$ *vs.* pcDNA3.1+miR-29b mimics group, "&" $p<0.05$ *vs.* pcHULC+NC). **D,** Western blot bands and gray value analysis of inflammatory cytokines (IL-1, IL-6 and IL-8) in HUVEC cells ("*" $p<0.05$ *vs.* pcDNA3.1+NC group, "#" $p<0.05$ *vs.* pcDNA3.1+miR-29b mimics group, "&" $p<0.05$ *vs.* pcHULC+NC) **E,** EdU staining. (magnification: 400×) **F,** Representative images of capillary-like structures and quantitative analysis of the total tube length (magnification: 40×) ("*" $p<0.05$ *vs.* pcDNA3.1+NC group, "#" $p<0.05$ *vs.* pcDNA3.1+miR-29b mimics group, "&" $p<0.05$ *vs.* pcHULC+NC).

This research adds to the growing body of evidence implicating miRNAs in the regulation of angiogenesis. Based on these data, it was speculated that miR-29b may become a potential therapeutic target for heart neovascularization. This assumption requires to be validated by further experiments and more clinical trials.

Conclusions

Summarily, lncRNA HULC protects HUVEC cells from hypoxia-induced inflammatory damage by interacting with miR-29b, inhibiting its expression, while promoting angiogenesis. These findings indicate that lncRNA HULC may be an important regulator in the pathogenesis of MI, and it may also be a new biomarker for the diagnosis and treatment of MI.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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