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MALAT1 affects hypoxia-induced vascular endothelial cell injury and autophagy by regulating miR-19b-3p/HIF-1α axis

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Abstract

Cardiovascular disease has become the leading cause of death in the world. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) plays an important role in cardiovascular disease, such as stroke. However, the role of MALAT1 in hypoxia (HYP)-induced vascular endothelial cells (VECs) remains unclear. In the present study, HYP-treated human umbilical vein endothelial cells (HUVECs) were utilized to simulate HYP-induced VEC injury. It was found that after HYP treatment, the levels of MALAT1 and hypoxia-induced factor-1 (HIF-1α) in HUVECs were upregulated, while the level of miR-19b-3p was downregulated. Knockdown of MALAT1 with siRNA significantly reduced the HIF-1α level induced by HYP. In addition, MALAT1 knockdown inhibited HYP-induced HUVECs apoptosis, autophagy and inflammation. The overexpression of HIF-1α overcame the effect of MALAT1 knockdown. Mechanism analysis showed that MALAT1-targeted miR-19b-3p and then regulated downstream HIF-1α. MALAT1 knockdown increased the level of miR-19b-3p in cells, and increased miR-19b-3p further inhibited the expression of HIF-1α, thereby reducing the HYP-induced HUVECs apoptosis, autophagy and inflammation. Taken together, these results suggest that MALAT1 may be a potential target for mitigating HYP-induced endothelial cell injury.

Keywords Metastasis associated lung adenocarcinoma transcript 1 · miR-19b-3p · Hypoxia inducible factor-1α · Apoptosis · Autophagy · Inflammation

Introduction

Abnormalities of the heart or blood vessels lead to the development of cardiovascular disease [1]. In recent years, cardiovascular diseases have been the leading cause of death in the world. Hypoxia (HYP) induces various stress responses in endothelial cells, such as cell proliferation [2], migration [3], inflammation [4], and apoptosis [5]. Vascular endothelial cells (VECs) are the most common cells in the heart and cerebrovascular that play an important role in the process of hypoxic heart injury [6]. Myocardial HYP-induced endothelial cells apoptosis can cause myocardial dysfunction, such as heart failure, myocardial ischemia and myocardial infarction (MI) [7]. Therefore, it is of crucial significance to investigate the molecular mechanism of HYP-induced VECs differentiation for the treatment of cardiovascular diseases.

Long non-coding RNAs are involved in many biological effects associated with human diseases, such as autophagy, apoptosis, and inflammation [8, 9]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) plays an important role in cardiovascular disease [10, 11]. Studies have shown that MALAT1 was significantly elevated during HYP and controlled the phenotypic transition of endothelial cells [12]. Additionally, MALAT1 also promoted pyroptosis of human endothelial cells [13], regulated angiogenesis [14, 15], autophagy [16] and inflammation [17]. However, the role of MALAT1 in HYP-induced VECs is still unknown.

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MicroRNAs (miRNAs, 18–22 nt) play an important role in a variety of genes and cellular processes by binding to the 3′ non-coding region (3′UTR). MiR-19b-3p is abnormally expressed in various cancers, such as clear cell renal cell carcinoma [18], melanoma [19], lung cancer [20], and breast cancer [21]. It has been reported that miR-19b-3p associates with HYP adaptation. Under HYP conditions, miR-19b-3p could induce apoptosis of great tit embryonic fibroblasts and regulate cell cycle [22]. However, whether miR-19b-3p can respond to hypoxic-induced VEC injury is far from being fully revealed.

Hypoxia inducible factor-1 (HIF-1) and its associated signaling pathways play an important role in HYP-induced injury [23]. HIF-1 α is a HIF subunit existed in the cytoplasm, which associated the response to oxidative stress [24]. HYP-induced endothelial cell injury and apoptosis by upregulating the expression of endothelin-1 (ET-1) and HIF-1α [25]. It has been found that HIF-1α was involved in the angiogenesis of myocardial infarction [26].

In the present study, we investigated the role of MALAT1 in hypoxic-induced VEC injury and the corresponding molecular mechanism. The results suggested that MALAT1 affected HYP-induced VEC injury and autophagy by regulating miR-19b-3p/HIF-1α axis.

Materials and methods

Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) were purchased from the American Type Culture Collection (ATCC® PCS-100–013™, Manassas, VA) and stored in DMEM medium supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) and 1% penicillin/streptomycin. The cells were cultured at 37 °C for 0, 6, 12 or 24 h in an airtight modular incubator (Billups-Rothenberg, San Diego, California, USA) under hypoxic conditionss (1% O_2, 5% CO_2 and 94% N_2). All transfection was conducted performed using lipofectamine-2000 according to the manufacturer's instructions.

RT-qPCR

HUVECs were incubated at 37 °C for 0, 6, 12 or 24 h under hypoxic conditions. The mRNA level of MALAT1, miR-19b-3p and HIF-1α was evaluated by RT-qPCR. Total RNA was isolated from HUVECs using TRIzol® reagent (Takara Bio, Inc., Otsu, Japan) and reverse-transcribed into cDNA using Revert Aid first-strand cDNA synthesis Kit (Thermo Fisher Scientific, Guangzhou, China). qPCR was performed using BioRad CFX96 Sequence Detection System (BioRad, Berkeley, CA, USA) with SYBR Premix ExTaq II (Takara, Dalian, China) according to the instructions of the manual. β-ACTIN was employed as an internal reference, and the mRNA level of MALAT1, miR-19b-3p and HIF-1α was evaluated with $2^{-\Delta\Delta C_t}$ method. Primer were as follows:

MALAT1: forward 5′-TGCAATGCACTCAGCATGC-3′, reverse 5′-CCGACATTACGACGTATTCG-3′;
miR-19b-3p: forward 5′-TGCTAACGATGTACTACG CG-3′, reverse 5′-TACTTACGCTGCTGCCATGC-3′;
HIF-1α: forward 5′-ATGGCTCGAACCGCTCAGT-3′, reverse 5′-CTCGAGAACTGCTGCTACG-3′;
B-ACTIN: forward 5′-GCCTGTGTCACTCGCTACGT-3′, reverse 5′-GGCTACTCGACTCGATCGCG-3′.

Western blot

The protein levels of HUVECs treated with HYP were measured using western blotting. The cells were lysed with prechilled RIPA buffer (Thermo Scientific, Guangzhou, China) supplemented with a protease inhibitor. Nucleocapsid protein was extracted using the Nuclear and Cytoplasmic Protein Extraction Kit according to the manufacturer's instructions (Beyotime, Shanghai, China). Then, the protein was then isolated with 10% SDS-PAGE and transferred to the PDFV membrane (BioRad, Beijing, China). Thereafter, the membrane was incubated with blocking buffer and combined with primary antibodies for LaminB (#13435, 1:1000, Cell Signaling Technology, Beijing, China), HIF-1α (#36169, 1:1000, CST, Beijing, China), Cleaved caspase 3 (#9654, 1:1000, CST, Beijing, China), LC3II/I (ab51520, 1:5000, Abcam, Beijing, China), p-p62 (ab155686, 1:1000, 1:5000, Abcam, Beijing, China), Bcl-2 (#3498, 1:1000, CST, Beijing, China), Bax (#2772, 1:1000, CST, Beijing, China), p65 (#8242, 1:1000, CST, Beijing, China), and GAPDH (#5174, 1:1000, CST, Beijing, China). Subsequently, the primary antibody-incubated membrane was then incubated with corresponding secondary antibodies (Beijing Dingguo Changsheng Biotechnology Co, Ltd, Lincoln, NE). Then, the bands were visualized with densitometry (BioRad, Hercules, CA). Protein levels were quantified by Image-Pro Plus.

MALAT1 knockdown

MALAT1-specific siRNAs (si-MALAT1-1, si-MALAT1-2 and si-MALAT1-3) were designed and synthesized by Thermo Fisher Scientific (Guangzhou, China). Then, siRNAs were introduced into pEnter4-N-Flag (Addgene, Wuhan, China) to construct recombinant plasmids (pEnter-si-MALAT1-1, pEnter-si-MALAT1-2 and pEnter-si-MALAT1-3). Then, according to the manufacturer's instructions, three recombinant plasmids were transfected into HUVECs that had been HYP-treated for 24 h with lipofectamine-2000 to knockdown MALAT1.

Hoechst 33258 staining

HUVECs were fixed with 4% paraformaldehyde and stained with 5 mg/L Hoechst 33258 for 30 min at 37 °C. The enriched and ruptured apoptotic cells were then examined with a fluorescent microscope (TE-2000 'Nikon' Japan).

ELISA

The levels of ROS, TNF-α and IL-6 in HUVECs were measured using the corresponding ELISA kits according to the manufacturer's instructions (CUSABIO, Shanghai, China).

Immunofluorescence assay

Nuclear translocation of NF-κB p65 was detected by immunofluorescence analysis. Briefly, HUVECs were fixed with 4% formaldehyde, sealed with 0.3% Triton™ X-100, and incubated overnight with anti-NF-κB p65 (#8242, 1:400 dilution, CST, Beijing, China) at 4 °C. The cells were then washed with PBS, incubated with anti-Rabbit IgG (H + L) (1:500 dilution, CST, Beijing, China) at 37 °C for 40 min, and stained with DAPI (#8961, CST, Beijing, China). Images were generated using a laser confocal scanning microscope and fluorescent intensity was calculated using Leica Application Suite Advanced Fluorescence 4.0.

Determination of autophagy activity

GFP-LC3 plasmid was implemented to detect autophagy activity. Briefly, HUVECs were transfected with GFP-LC3 using FuGENE HD® Transfection Reagent according to the manufacturer's instructions (Sigma, MO, USA). After transfection for 24 h, the transfection efficiency was assessed by fluorescence microscopy. The average number of puncta was equal to autophagy activity. The experiment was repeated five times in each group, and the data were used to compare the groups.

Pull-down assay

HUVECs were cleaved and incubated with biotin-labeled DNA oligomers corresponding to MALAT1. The mixture was incubated with streptavidin-coupled agarose beads (Invitrogen) for 4 h at 4 °C. The beads were washed and resuspended in TRIzol to extract RNA for qPCR.

Dual luciferase reporter assay

The target sites between MALAT1, miR-19b-3p and HIF-1α were predicted with miRDB and TargetScan. The target relationship between miR-19b-3p and HIF-1α was verified by Dual luciferase reporter assay. Briefly, HIF-1α-wt and HIF-1α-mut were introduced into luciferase reporter plasmid pGMERSE-Lu (Genomedtech, Shanghai, China) to construct recombinant plasmids (pGMERSE-HIF-1α-wt and pGMERSE-HIF-1α-mut). The recombinant plasmids were then co-transfected into HUVEC with miR-19b-3p or miR-NC using Lipofectamie 2000 (Invitrogen, Beijing, China). After transfection for 48 h, luciferase activity was measured using the Dual-Luciferase Reporter assay system (Promega, Madison, WI).

Statistical analysis

Results are expressed as the mean ± SD. Statistical analyzes was performed using GraphPad Prism 5 (San Diego, CA, USA). Differences between groups were assessed by one-way ANOVA and Newman–Keuls multiple comparison test. $p < 0.05$, the difference was statistically significant.

Results

MALAT1 knockdown inhibited HYP-induced injury and autophagy by suppressing HIF-1α expression

First, the role of MALAT1 in HYP-induced VEC injury was investigated. As shown in Fig. 1a, the level of HIF-1α in HUVECs was increased in a time-dependent manner after HYP treatment for 6, 12 and 24 h, respectively. Similarly, the level of MALAT1 in HUVECs was further increased in a time-dependent manner under the same treatment (Fig. 1b). When MALAT1 was knocked down with three different siRNA, the level of MALAT1 in cells was significantly reduced, indicating that siRNA could effectively knock MALAT1 down (Fig. 1c). Besides, we found that HIF-1α in the si-MALAT1 + HYP group was significantly lower in mRNA and protein level than that in the HYP group and the si-NC + HYP group (Fig. 1d, e). Besides, the present study found that the low expression of MALAT1 reversed the HYP-induced increase in ROS level and autophagy activity (Fig. 1f–i). However, no significant change was observed in the si-NC + HYP group compared with the HYP group. Notably, HIF-1α agonist ($CoCl_2$) overcame the effects of MALAT1 knockdown (Fig. 1j–l). These results indicate that MALAT1 knockdown inhibits HYP-induced injury and autophagy by suppressing HIF-1α expression.

MALAT1 knockdown-inhibited HYP-induced apoptosis and inflammation by inhibiting HIF-1α expression

Studies found that low expression of MALAT1 reversed HYP-induced increases in Bax and cleaved caspase 3, as well as reduction in Bcl-2 (Fig. 2a–c). As expected,

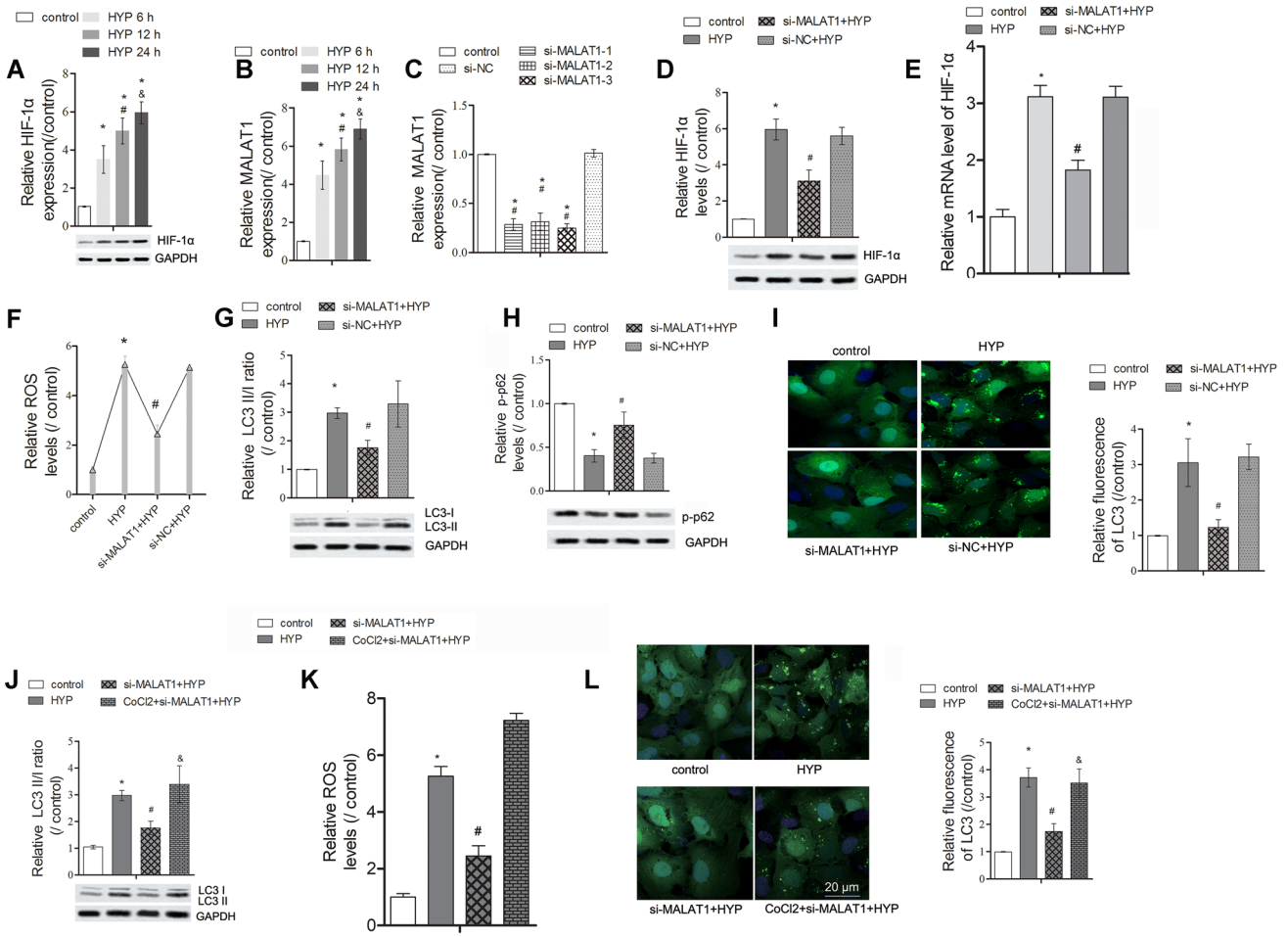

Fig. 1 MALAT1 knockdown inhibited HYP-induced injury and autophagy by suppressing HIF-1α expression. **a, b** HUVECs were incubated at 37 °C for 0, 6, 12 or 24 h under hypoxic conditions (1% O_2, 5% CO_2 and 94% N_2). **a** The protein level of HIF-1α was measured by western blotting (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP 6 h; $^{\&}p<0.05$ vs HYP 12 h). **b** The mRNA level of MALAT1 was measured by RT-qPCR (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP 6 h; $^{\&}p<0.05$ vs HYP 12 h). **c** HUVECs were transfected with si-NC, si-MALAT1-1, si-MALAT1-2 and si-MALAT1-3, respectively. The mRNA level of MALAT1 was measured by RT-qPCR (*$p<0.05$ vs control; $^{\#}p<0.05$ vs si-NC). **d–h** HUVECs were divided into four groups: Control group, HYP group, si-MALAT1 + HYP group, si-NC + HYP group. **d** The protein level of HIF-1α was measured by western blotting. GAPDH was used as an internal reference (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **e** The mRNA level of HIF-1α was measured by q-PCR. GAPDH was used as an internal reference (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **f** ROS production was detected by ELISA assay (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **g, h** The protein levels of LC3II/I and p-p62 were measured by western blotting (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **i** Autophagy activity was determined by immunofluorescence assay (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **j, k** HUVECs were divided into four groups: Control group, HYP group, si-MALAT1 + HYP group, $CoCl_2$ (150 μM) + si-MALAT1 + HYP group. **j** The protein level of LC3II/I was measured by western blotting (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP; $^{\&}p<0.05$ vs si-MALAT1 + HYP). **k** Autophagy activity was determined by immunofluorescence assay (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP; $^{\&}p<0.05$ vs si-MALAT1 + HYP)

Hoechst staining showed that HYP-induced HUVECs apoptosis (Fig. 2d). No significant change was observed in the si-NC + HYP group. Notably, HIF-1α agonist ($CoCl_2$) overcame the effects of MALAT1 knockdown (Fig. 2e). In addition, HYP also increased the level of inflammatory factors (TNF-α and IL-6) in HUVECs and promoted nuclear translocation of p65. However, MALAT1 knockdown apparently counteracted the effect of HYP on cellular inflammation. No significant change was observed in the si-NC + HYP group (Fig. 2f–h). Likewise, HIF-1α agonist ($CoCl_2$) overcame the effects of MALAT1 knockdown (Fig. 2i). These results demonstrate that MALAT1 knockdown inhibited HYP-induced apoptosis and inflammation by inhibiting HIF-1α expression.

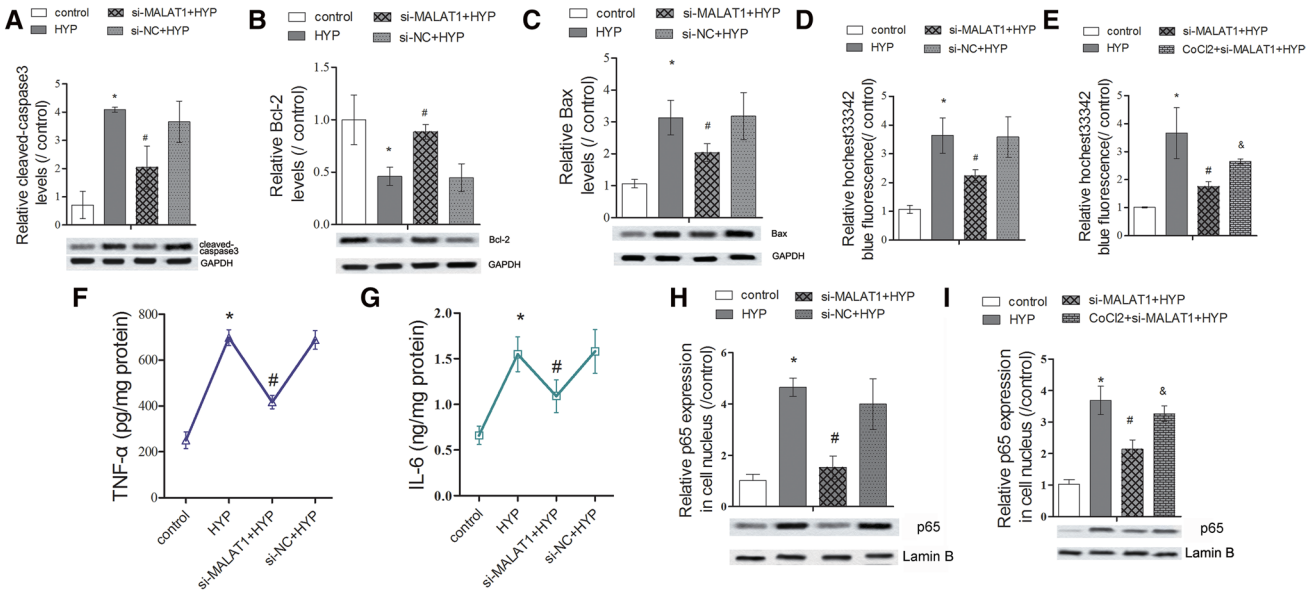

Fig. 2 MALAT1 knockdown inhibited HYP-induced apoptosis and inflammation by inhibiting HIF-1α expression. HUVECs were divided into four groups: Control group, HYP group, si-MALAT1 + HYP group, si-NC + HYP group. **a–c** The protein levels of cleaved caspase 3, Bcl-2 and Bax were measured by western blotting. GAPDH was employed as an internal reference (*$p < 0.05$ vs control; #$p < 0.05$ vs HYP). **d** Apoptosis was measured by Hochest 3342 staining (*$p < 0.05$ vs control; #$p < 0.05$ vs HYP). **e** HUVECs were divided into four groups: Control group, HYP group, si-MALAT1 + HYP group, $CoCl_2$ (150 μM) + si-MALAT1 + HYP group. Apoptosis was measured by Hoechst 3342 staining (*$p < 0.05$ vs control; #$p < 0.05$ vs HYP; &$p < 0.05$ vs si-MALAT1 + HYP). **f, g** The levels of inflammatory cytokines (TNF-α and IL-6) were measured by ELISA assay (*$p < 0.05$ vs control; #$p < 0.05$ vs HYP). **h** The protein level of p65 in the nucleus was measured by western blotting. LaminB was employed as an internal reference (*$p < 0.05$ vs control; #$p < 0.05$ vs HYP). **i** HUVECs were divided into four groups: Control group, HYP group, si-MALAT1 + HYP group, $CoCl_2$ (150 μM) + si-MALAT1 + HYP group. The protein level of p65 in the nucleus was measured by western blotting. LaminB was employed as an internal reference (*$p < 0.05$ vs control; #$p < 0.05$ vs HYP; &$p < 0.05$ vs si-MALAT1 + HYP)

MALAT1 may partially regulate HYP-induced autophagy, apoptosis, and inflammation by targeting miR-19b-3p

The target of MALAT1 was predicted by miRDB (Fig. 3a). The level of miR-19b-3p was measured by RT-qPCR. As shown in Fig. 3b, the level of miR-19b-3p in HUVEC after HYP treatment was decreased in a time-dependent manner. However, low expression of MALAT1 significantly increased the level of miR-19b-3p in HUVECs (Fig. 3c). Pull-down assay showed that MALAT1 specifically regulated the expression of miR-19b-3p (Fig. 3d). Further analysis showed that miR-19b-3p overexpression partially reversed the HYP-induced decrease in p-p62 level and the increase in ROS level (Fig. 3e, f). Interestingly, miR-19b-3p overexpression partially rescued HYP-induced apoptosis and prevented p65 nuclear translocation (Fig. 3g, h). No significant change was observed in the mimic-NC + HYP group. These results suggest that MALAT1 may partially regulate HYP-induced autophagy, apoptosis, and inflammation by targeting miR-19b-3p.

miR-19b-3p regulated HYP-induced autophagy, apoptosis and inflammation by targeting HIF-1α

Downstream target protein of miR-19b-3p was predicted by TargetScan (Fig. 4a). The level of HIF-1α was measured by RT-qPCR. As shown in Fig. 4b, HIF-1α level was significantly decreased in HUVECs transfected with miR-19b-3p mimics, but no significant change was observed in the NC-mimics group. Dual luciferase reporter assay was further employed to verify the target relationship between miR-19b-3p and HIF-1α (Fig. 4c). Meanwhile, this study investigated the effects of miR-19b-3p and HIF-1α interaction on HUVECs autophagy, apoptosis and inflammation. As shown in Fig. 4d–f, miR-19b-3p mimics significantly inhibited the apoptosis and autophagy of HUVECs induced by HYP, and prevented the nuclear translocation of p65. The HIF-1α agonist ($CoCl_2$) counteracted the effects of miR-19b-3p mimics. These results suggest that miR-19b-3p regulates HYP-induced autophagy, apoptosis, and p65 nuclear translocation by targeting HIF-1α.

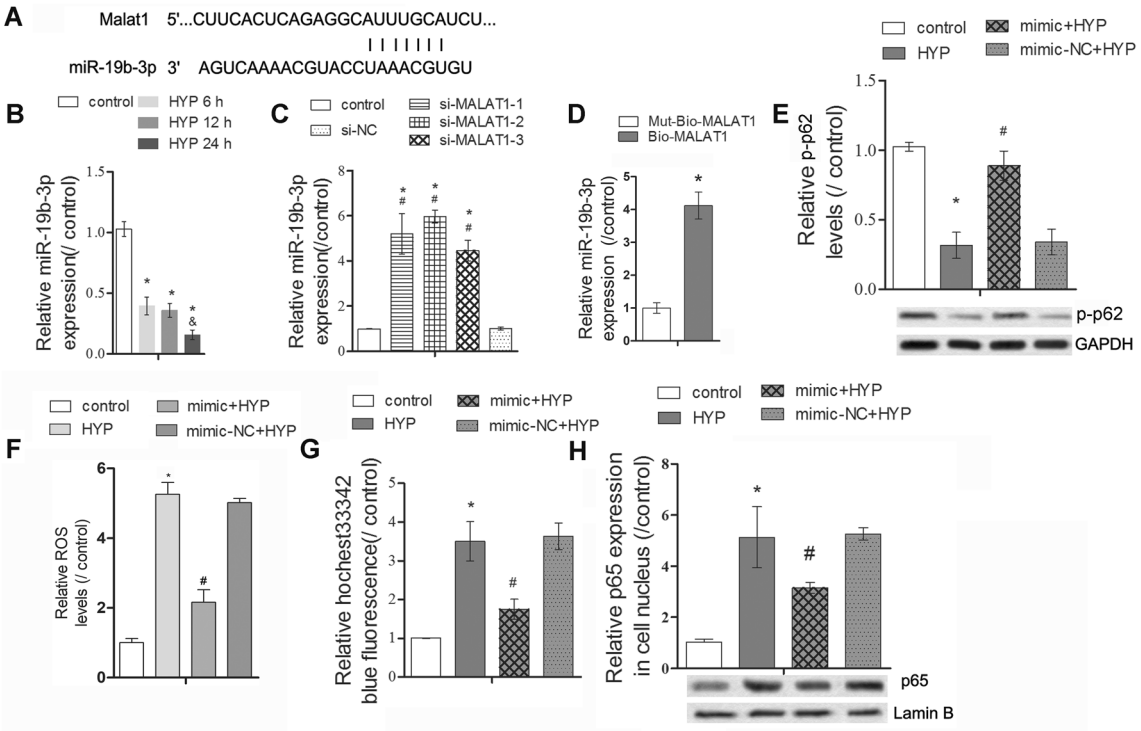

Fig. 3 MALAT1 may partially regulate HYP-induced autophagy, apoptosis, and inflammation by targeting miR-19b-3p. **a** The target site between MALAT1 and miR-19b-3p was predicted by miRDB. **b** HUVECs were incubated at 37 °C for 0, 6, 12 or 24 h under hypoxic conditions (1% O_2, 5% CO_2 and 94% N_2). The mRNA level of miR-19b-3p was measured by RT-qPCR (*$p<0.05$ vs control; $^{\&}p<0.05$ vs HYP 12 h). **c** HUVECs were transfected with si-NC, si-MALAT1-1, si-MALAT1-2 and si-MALAT1-3, respectively. The mRNA level of miR-19b-3p was measured by RT-qPCR (*$p<0.05$ vs control; $^{\#}p<0.05$ vs si-NC). **d** The target relationship was further confirmed by pull-down assay (*$p<0.05$ vs Mut-Bio-MALAT1). **e** The protein level of p-p62 was measured by western blotting. GAPDH was employed as an internal reference (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **f** ROS production was detected by ELISA assay (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **g** Apoptosis was measured by Hochest 3342 staining (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP). **h** The protein level of p65 in the nucleus was measured by western blotting. LaminB was employed as an internal reference (*$p<0.05$ vs control; $^{\#}p<0.05$ vs HYP)

Discussion

HYP plays an important role in cardiovascular diseases (CVDs) such as MI, CF and CHD [7, 27]. Vascular endothelial dysfunction caused by myocardial HYP is related to the pathogenesis of several cardiovascular diseases [28]. VECs are the cells that respond directly to HYP [29]. Numerous studies suggest that it is essential to protect VECs from HYP-induced injury [30–32]. Lee et al. confirmed that Ang II participated in the occurrence of cardiovascular disease by inducing lipid peroxidation in human VECs [33]. Chang et al. reported that root extract could prevent endothelial cell death and apoptosis caused by HYP and protect cells from oxidative stress [34]. In the present study, HUVECs under hypoxic conditions were applied to simulate HYP-induced VEC injury. The result showed that the low expression of lncRNA MALAT1 could alleviate the HUVECs injury induced by HYP by targeting the regulation of miR-19b-3p/HIF-1α axis.

It has been reported that MALAT1 is closely related to cell function [10, 35]. Wang and Zhou reported that MALAT1 promoted the inflammatory response of microglia cells through the MyD88/IRAK1/TRAF6 pathway [36]. Similarly, MALAT1 was highly expressed in HUVECs exposed to HYP in this study, which reduced the level of miR-19b-3p in cells and activated the expression of HIF-1α. MALAT1 knockdown significantly attenuated HYP-induced HUVECs apoptosis, autophagy and p65 nuclear translocation. MiR-19b-3p mimics showed the same effect, while the HIF-1α activator CoCl2 showed the opposite effect. Collectively, these results demonstrate that MALAT1 promoted the injury of VECs induced by HYP.

Recent studies have suggested that miR-19b-3p plays an important in acute myocardial infarction and myocardial fibrosis [37, 38]. Xue et al. found that miR-19b-3p targeted and negatively regulated peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), thereby inducing mitochondrial dysfunction and apoptosis [39]. Wang et al. found that the levels of miR-19b-3p, miR-134-5p and miR-186-5p

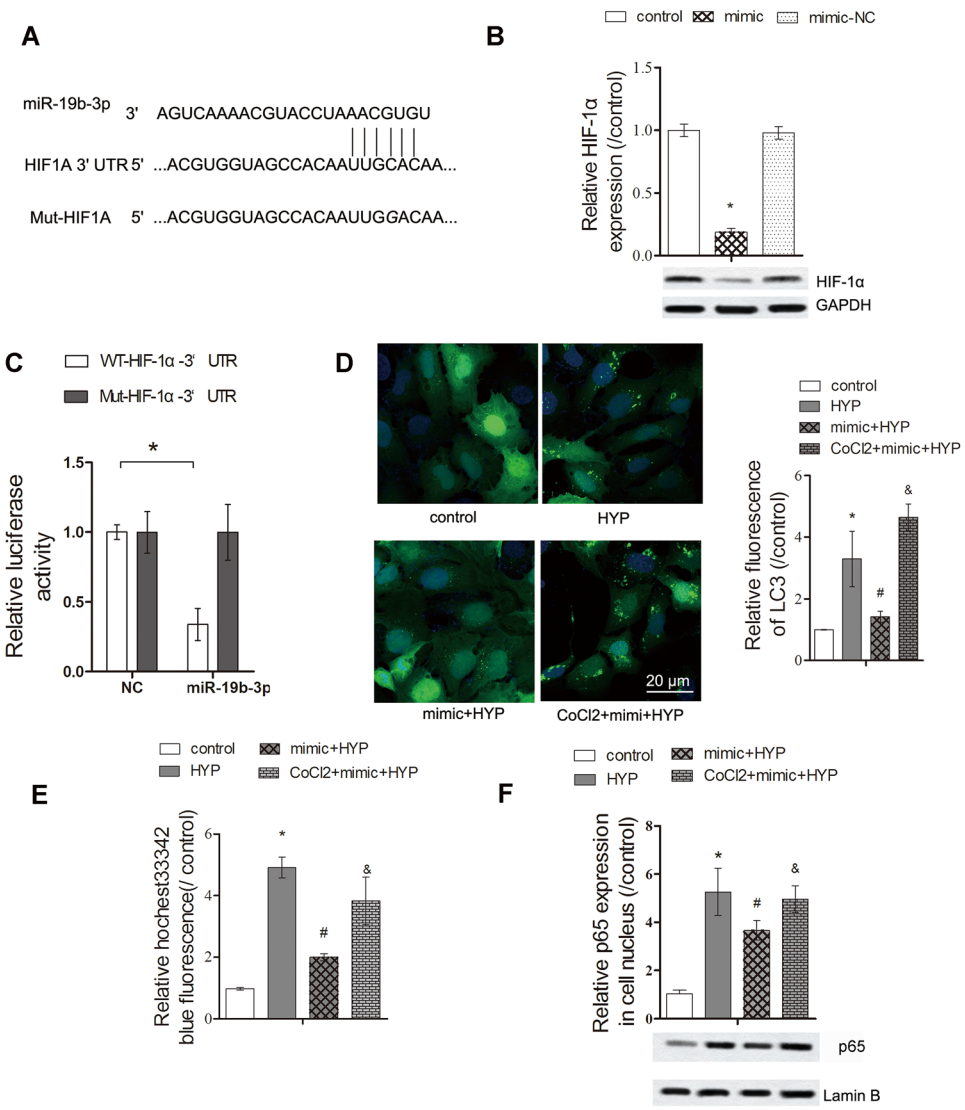

Fig. 4 miR-19b-3p regulated HYP-induced autophagy, apoptosis and inflammation by targeting HIF-1α. **a** The target site between miR-19b-3p and HIF-1α. **b** HUVECs were transfected with mimic-NC or miR-19b-3p mimic. The protein level of HIF-1α was measured by western blotting. GAPDH was employed as an internal reference (*$p < 0.05$ vs control). **c** Target relationship was further confirmed by the dual luciferase reporter assay (*$p < 0.05$ vs NC + WT-HIF-1α-3′UTR). **d–f** HUVECs were divided into four groups: Control group, HYP group, mimic + HYP group, $CoCl_2$ (150 μM) + mimic + HYP group. **d** Autophagy activity was determined by immunofluorescence assay (*$p < 0.05$ vs control; $^{\#}p < 0.05$ vs HYP; $^{\&}p < 0.05$ vs mimic + HYP). **e** Apoptosis was measured by Hoechst 3342 staining (*$p < 0.05$ vs control; $^{\#}p < 0.05$ vs HYP; $^{\&}p < 0.05$ vs mimic + HYP). **f** The protein level of p65 in the nucleus was measured by western blotting. LaminB was employed as an internal reference (*$p < 0.05$ vs control; $^{\#}p < 0.05$ vs HYP; $^{\&}p < 0.05$ vs mimic + HYP)

in the early stage of acute myocardial infarction (AMI) were significantly increased, which could be used as new markers for early diagnosis of AMI [38]. However, this study found that miR-19b-3p was downregulated in HYP-treated HUVECs. The overexpression of miR-19b-3p significantly inhibited HYP-induced HUVECs apoptosis, autophagy, and p65 nuclear translocation by reducing HIF-1α level. This study suggests that miR-19b-3p may be used as a novel vascular protectant in the future.

HIF-1 is a key transcription factor for HYP adaptation [40]. HIF-1 overexpression is associated with inflammation and HYP-induced endothelial cell injury [41]. HIF-1α is rapidly degraded by acrylated hydroxylase under normal oxygen and is abnormally accumulated during acute HYP [42, 43]. Current studies have shown that HIF-1α level was increased significantly in mRNA and protein levels in HUVECs after HYP treatment. Besides, further mechanism analysis indicated that MALAT1 promoted HYP-induced HUVECs autophagy, apoptosis and p65 nuclear translocation by targeting the adsorption of miR-19b-3p and upregulation of HIF-1α expression. In addition, inflammatory cytokines (TNF-α and IL-1β) can also induce HIF-1α accumulation [44]. We found that HYP-induced increases in TNF-α and IL-1β further promoted the accumulation of HIF-1α in HUVECs, thus aggravating HYP-induced VEC injury.

Autophagy plays an important role in cardiovascular diseases, such as inhibiting myocardial remodeling [45], improving myocardial function [46], regulating advanced plaques of AS [47] and responding to cardiac stress [48]. Wang et al. reported that MALAT1 enhanced the expression of Beclin-1 through adsorption of miR-216a-5p to neutralize the inhibitory effect of miR-216-5p on autophagy and survival of cells [16]. Duan et al. found that PM could reduce hypertension, atherosclerosis and myocardial infarction by down-regulating the level of miR-19a-3p [49]. HYP-induced

autophagy of tumor cells by upregulating the level of HIF-1α. Huang et al. believed that the HIF-1α/miR-224-3p/ATG5 axis affected cell motility and chemotherapeutic sensitivity by regulating HYP-induced autophagy in glioblastomas and astrocytomas [50]. Consistent with these results, we found that HYP induced the expression of HIF-1α, and the overexpression of HIF-1α promoted ROS accumulation and autophagy. Notably, low expression of HIF-1α abolished the promotional effect. After MALAT1 was knocked down by siRNA, the level of miR-19b-3p in the cells was significantly increased, while the level of HIF-1α was significantly decreased. Reduced HIF-1α further inhibited HYP-induced autophagy in HUVECs. In short, these results indicate that si-MALAT1 inhibit HYP-induced autophagy by upregulating the level of HIF-1α via targeting miR-19a-3p.

Conclusion

In conclusion, current studies have explored the effects of MALAT1 on HYP-induced VEC injury and its potential molecular mechanisms. The results showed that low expression of MALAT1 suppressed HYP-induced HUVECs apoptosis, autophagy and p65 nuclear translocation by regulating miR-19b-3p/HIF-1α axis. Collectively, these results demonstrate that MALAT1 may be a potential target for relieving HYP-induced endothelial cell injury.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Li B, Xu X, Wang X, Yu H, Li X, Tao W, Wang Y, Yang L (2012) A systems biology approach to understanding the mechanisms of action of chinese herbs for treatment of cardiovascular disease. Int J Mol Sci 13(10):13501–13520. https://doi.org/10.3390/ijms131013501
2. Cao H, Yu D, Yan X, Wang B, Yu Z, Song Y, Sheng L (2019) Hypoxia destroys the microstructure of microtubules and causes dysfunction of endothelial cells via the PI3K/Stathmin1 pathway. Cell Biosci 9(1):20. https://doi.org/10.1186/s13578-019-0283-1
3. Lin L, Li G, Zhang W, Wang YL, Yang H (2019) Low-dose aspirin reduces hypoxia-induced sFlt1 release via the JNK/AP-1 pathway in human trophoblast and endothelial cells. J Cell Physiol 234:18928–18941. https://doi.org/10.1002/jcp.28533
4. Cui C, Li Y, Liu Y (2019) Down-regulation of miR-377 suppresses high glucose and hypoxia-induced angiogenesis and inflammation in human retinal endothelial cells by direct up-regulation of target gene SIRT1. Hum Cell 32:260–274. https://doi.org/10.1007/s13577-019-00240-w
5. Wang HW, Jiang X, Zhang Y, Wang J, Xie J, Wang YQ, Li YH (2019) FGF21 protects against hypoxia injury through inducing HSP72 in cerebral microvascular endothelial cells. Front Pharmacol 10:101. https://doi.org/10.3389/fphar.2019.00101
6. Luo J, Martinez J, Yin X, Sanchez A, Tripathy D, Grammas P (2012) Hypoxia induces angiogenic factors in brain microvascular endothelial cells. Microvasc Res 83:138–145. https://doi.org/10.1016/j.mvr.2011.11.004
7. Jessica C, Lounsbury KM (2015) Hypoxia-mediated biological control. J Cell Biochem 112:735–744. https://doi.org/10.1002/jcb.22956
8. Wei H, Hu J, Pu J, Tang Q, Li W, Ma R, Xu Z, Tan C, Yao T, Wu X, Long X, Wang J (2019) Long noncoding RNA HAGLROS promotes cell proliferation, inhibits apoptosis and enhances autophagy via regulating miR-5095/ATG12 axis in hepatocellular carcinoma cells. Int Immunopharmacol 73:72–80. https://doi.org/10.1016/j.intimp.2019.04.049
9. Wan P, Su W, Zhang Y, Li Z, Deng C, Li J, Jiang N, Huang S, Long E, Zhuo Y (2019) LncRNA H19 initiates microglial pyroptosis and neuronal death in retinal ischemia/reperfusion injury. Cell Death Differ. https://doi.org/10.1038/s41418-019-0351-4
10. Sun R, Zhang L (2019) Long non-coding RNA MALAT1 regulates cardiomyocytes apoptosis after hypoxia/reperfusion injury via modulating miR-200a-3p/PDCD4 axis. Biomed Pharmacother 111:1036–1045. https://doi.org/10.1016/j.biopha.2018.12.122
11. Ruan W, Li J, Xu Y, Wang Y, Zhao F, Yang X, Jiang H, Zhang L, Saavedra JM, Shi L, Pang T (2019) MALAT1 up-regulator polydatin protects brain microvascular integrity and imeliorates stroke through C/EBPbeta/MALAT1/CREB/PGC-1alpha/PPARgamma pathway. Cell Mol Neurobiol 39:265–286. https://doi.org/10.1007/s10571-018-00646-4
12. Michalik KM, Xintian Y, Yosif M, Anuradha D, Martin ZR, Thomas B, David J, Yuliya P, Wei C, Shizuka U (2014) Long noncoding RNA MALAT1 regulates endothelial cell function and vessel growth. Circ Res 114:1389–1397. https://doi.org/10.1161/CIRCRESAHA.114.303265
13. Song Y, Yang L, Guo R, Lu N, Shi Y, Wang X (2019) Long noncoding RNA MALAT1 promotes high glucose-induced human endothelial cells pyroptosis by affecting NLRP3 expression through competitively binding miR-22. Biochem Biophys Res Commun 509:359–366. https://doi.org/10.1016/j.bbrc.2018.12.139
14. Huang XJ, Xia Y, He GF, Zheng LL, Cai YP, Yin Y, Wu Q (2018) MALAT1 promotes angiogenesis of breast cancer. Oncol Rep 40:2683–2689. https://doi.org/10.3892/or.2018.6705
15. Ren L, Wei C, Li K, Lu Z (2019) LncRNA MALAT1 up-regulates VEGF-A and ANGPT2 to promote angiogenesis in brain microvascular endothelial cells against oxygen-glucose deprivation via targetting miR-145. Biosci Rep 39(3):BSR20180226. https://doi.org/10.1042/bsr20180226
16. Wang K, Yang C, Shi J, Gao T (2019) Ox-LDL-induced lncRNA MALAT1 promotes autophagy in human umbilical vein endothelial cells by sponging miR-216a-5p and regulating Beclin-1 expression. Eur J Pharmacol 858:172338. https://doi.org/10.1016/j.ejphar.2019.04.019
17. Puthanveetil P, Chen S, Feng B, Gautam A, Chakrabarti S (2015) Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. J Cell Mol Med 19:1418–1425. https://doi.org/10.1111/jcmm.12576
18. Wang L, Yang G, Zhao D, Wang J, Bai Y, Peng Q, Wang H, Fang R, Chen G, Wang Z, Wang K, Li G, Yang Y, Wang Z, Guo P, Peng L, Hou D, Xu W (2019) CD103-positive CSC exosome promotes EMT of clear cell renal cell carcinoma: role of remote MiR-19b-3p. Mol Cancer 18(1):86. https://doi.org/10.1186/s12943-019-0997-z

19. Wei YP, Wang XH, Liu G, Zhang JF, Yang YX, Zhang J, Song XL, Li ZD, Zhao LD (2018) Matrine exerts inhibitory effects in melanoma through the regulation of miR-19b-3p/PTEN. Int J Oncol 53:791–800. https://doi.org/10.3892/ijo.2018.4414
20. Bulgakova O, Zhabayeva D, Kussainova A, Pulliero A, Izzotti A, Bersimbaev R (2018) miR-19 in blood plasma reflects lung cancer occurrence but is not specifically associated with radon exposure. Oncol Lett 15:8816–8824. https://doi.org/10.3892/ol.2018.8392
21. Maleki E, Ghaedi K, Shahanipoor K, Karimi Kurdistani Z (2018) Down-regulation of microRNA-19b in hormone receptor-positive/HER2-negative breast cancer. APMIS 126:303–308. https://doi.org/10.1111/apm.12820
22. Chen X, Qu Y, Cheng Y, Wang J, Lei X, Song G, Zhang H, Wang H, Lei F (2018) MiR-19b-3p regulates MAPK1 expression in embryonic fibroblasts from the great tit (*Parus major*) under hypoxic conditions. Cell Physiol Biochem 46:546–560. https://doi.org/10.1159/000488621
23. Jing L, Shao J, Sun W, Lan T, Jia Z, Ma H, Wang H (2019) Protective effects of two novel nitronyl nitroxide radicals on heart failure induced by hypobaric hypoxia. Life Sci. https://doi.org/10.1016/j.lfs.2019.05.037
24. Byun Y, Choi YC, Jeong Y, Lee G, Yoon S, Jeong Y, Yoon J, Baek K (2019) MiR-200c downregulates HIF-1alpha and inhibits migration of lung cancer cells. Cell Mol Biol Lett 24:28. https://doi.org/10.1186/s11658-019-0152-2
25. Feng Y, Li Q, Wu Y, Zhao N, Li L, Li L, Zhao L (2019) Blocking C/EBP beta protects vascular endothelial cells from injury induced by intermittent hypoxia. Sleep Breath 23:953–962. https://doi.org/10.1007/s11325-018-1759-7
26. Du Y, Ge Y, Xu Z, Aa N, Gu X, Meng H, Lin Z, Zhu D, Shi J, Zhuang R, Wu X, Wang X, Yang Z (2018) Hypoxia-inducible factor 1 alpha (HIF-1alpha)/vascular endothelial growth factor (VEGF) pathway participates in angiogenesis of myocardial infarction in muscone-treated mice: preliminary study. Med Sci Monit 24:8870–8877. https://doi.org/10.12659/msm.912051
27. Cheng F, Lan J, Xia W, Tu C, Chen B, Li S, Pan W (2016) Folic acid attenuates vascular endothelial cell injury caused by hypoxia via the inhibition of ERK1/2/NOX4/ROS pathway. Cell Biochem Biophys 74:205–211. https://doi.org/10.1007/s12013-016-0723-z
28. Liu B, Che W, Xue J, Zheng C, Tang K, Zhang J, Wen J, Xu Y (2013) SIRT4 prevents hypoxia-induced apoptosis in H9c2 cardiomyoblast cells. Cell Physiol Biochem 32:655–662. https://doi.org/10.1159/000354469
29. Zhang X, Liu S, Weng X, Zeng S, Yu L, Guo J, Xu Y (2018) Brg1 deficiency in vascular endothelial cells blocks neutrophil recruitment and ameliorates cardiac ischemia-reperfusion injury in mice. Int J Cardiol 269:250–258. https://doi.org/10.1016/j.ijcard.2018.07.105
30. Xiao X, Xu S, Li L, Mao M, Wang J, Li Y, Wang Z, Ye F, Huang L (2017) The effect of velvet antler proteins on cardiac microvascular endothelial cells challenged with ischemia-hypoxia. Front Pharmacol 8:601. https://doi.org/10.3389/fphar.2017.00601
31. Tsai HH, Lin CP, Lin YH, Hsu CC, Wang JS (2016) High-intensity Interval training enhances mobilization/functionality of endothelial progenitor cells and depressed shedding of vascular endothelial cells undergoing hypoxia. Eur J Appl Physiol 116:2375–2388. https://doi.org/10.1007/s00421-016-3490-z
32. Zhang Q, Shang M, Zhang M, Wang Y, Chen Y, Wu Y, Liu M, Song J, Liu Y (2016) Microvesicles derived from hypoxia/reoxygenation-treated human umbilical vein endothelial cells promote apoptosis and oxidative stress in H9c2 cardiomyocytes. BMC Cell Biol 17:25. https://doi.org/10.1186/s12860-016-0100-1
33. Lee SH, Fujioka S, Takahashi R, Oe T (2019) Angiotensin II-induced oxidative stress in human endothelial cells: modification of cellular molecules through lipid peroxidation. Chem Res Toxicol 32:1412–1422. https://doi.org/10.1021/acs.chemrestox.9b00110
34. Chang PK, Yen IC, Tsai WC, Chang TC, Lee SY (2018) Protective effects of *Rhodiola crenulata* extract on hypoxia-induced endothelial damage via regulation of AMPK and ERK pathways. Int J Mol Sci 19:2286. https://doi.org/10.3390/ijms19082286
35. Wang Q, Lu G, Chen Z (2019) MALAT1 promoted cell proliferation and migration via MALAT1/miR-155/MEF2A pathway in hypoxia of cardiac stem cells. J Cell Biochem 120:6384–6394. https://doi.org/10.1002/jcb.27925
36. Wang LQ, Zhou HJ (2018) LncRNA MALAT1 promotes high glucose-induced inflammatory response of microglial cells via provoking MyD88/IRAK1/TRAF6 signaling. Sci Rep 8:8346. https://doi.org/10.1038/s41598-018-26421-5
37. Fang L, Ellims AH, Moore XL, White DA, Taylor AJ, Chin-Dusting J, Dart AM (2015) Circulating microRNAs as biomarkers for diffuse myocardial fibrosis in patients with hypertrophic cardiomyopathy. J Transl Med 13:314. https://doi.org/10.1186/s12967-015-0672-0
38. Wang KJ, Zhao X, Liu YZ, Zeng QT, Mao XB, Li SN, Zhang M, Jiang C, Zhou Y, Qian C, Feng KG, Guan HQ, Tang TT, Cheng X, Chen ZJ (2016) Circulating MiR-19b-3p, MiR-134-5p and MiR-186-5p are promising novel biomarkers for early diagnosis of acute myocardial infarction. Cell Physiol Biochem 38:1015–1029. https://doi.org/10.1159/000443053
39. Xue Y, Wei Z, Ding H, Wang Q, Zhou Z, Zheng S, Zhang Y, Hou D, Liu Y, Zen K, Zhang CY, Li J, Wang D, Jiang X (2015) MicroRNA-19b/221/222 induces endothelial cell dysfunction via suppression of PGC-1alpha in the progression of atherosclerosis. Atherosclerosis 241:671–681. https://doi.org/10.1016/j.atherosclerosis.2015.06.031
40. Lambert CM, Roy M, Robitaille GA, Richard DE, Bonnet S (2010) HIF-1 inhibition decreases systemic vascular remodelling diseases by promoting apoptosis through a hexokinase 2-dependent mechanism. Cardiovasc Res 88:196–204. https://doi.org/10.1093/cvr/cvq152
41. Heikal L, Ghezzi P, Mengozzi M, Ferns G (2018) Assessment of HIF-1alpha expression and release following endothelial injury in-vitro and in-vivo. Mol Med 24:22. https://doi.org/10.1186/s10020-018-0026-5
42. Semenza GL (2014) Hypoxia-inducible factor 1 and cardiovascular disease. Annu Rev Physiol 76:39–56. https://doi.org/10.1146/annurev-physiol-021113-170322
43. Loboda A, Jozkowicz A, Dulak J (2012) HIF-1 versus HIF-2—is one more important than the other? Vascul Pharmacol 56:245–251. https://doi.org/10.1016/j.vph.2012.02.006
44. Gao L, Chen Q, Zhou X, Fan L (2012) The role of hypoxia-inducible factor 1 in atherosclerosis. J Clin Pathol 65:872–876. https://doi.org/10.1136/jclinpath-2012-200828
45. Wu X, He L, Chen F, He X, Cai Y, Zhang G, Yi Q, He M, Luo J (2014) Impaired autophagy contributes to adverse cardiac remodeling in acute myocardial infarction. PLoS ONE 9:e112891–e112891. https://doi.org/10.1371/journal.pone.0112891
46. Zhu H, Tannous P, Johnstone JL, Kong Y, Shelton JM, Richardson JA, Le V, Levine B, Rothermel BA, Hill JA (2007) Cardiac autophagy is a maladaptive response to hemodynamic stress. J Clin Invest 117:1782–1793. https://doi.org/10.1172/jci27523
47. Luo Y, Lu S, Zhou P, Ai QD, Sun GB, Sun XB (2016) Autophagy: an exposing therapeutic target in atherosclerosis. J Cardiovasc Pharmacol 67:266–274. https://doi.org/10.1097/fjc.0000000000000342
48. Mellor KM, Bell JR, Young MJ, Ritchie RH, Delbridge LM (2011) Myocardial autophagy activation and suppressed survival signaling is associated with insulin resistance in fructose-fed mice. J Mol Cell Cardiol 50:1035–1043. https://doi.org/10.1016/j.yjmcc.2011.03.002

49. Duan J, Yu Y, Li Y, Jing L, Yang M, Wang J, Li Y, Zhou X, Miller MR, Sun Z (2017) Comprehensive understanding of PM2.5 on gene and microRNA expression patterns in zebrafish (*Danio rerio*) model. Sci Total Environ 586:666–674. https://doi.org/10.1016/j.scitotenv.2017.02.042
50. Huang S, Qi P, Zhang T, Li F, He X (2019) The HIF-1α/miR-224-3p/ATG5 axis affects cell mobility and chemosensitivity by regulating hypoxia-induced protective autophagy in glioblastoma and astrocytoma. Oncol Rep 41:1759–1768. https://doi.org/10.3892/or.2018.6929