LINC00926 is involved in hypoxia-induced vascular endothelial cell dysfunction *via* miR-3194-5p regulating JAK1/STAT3 signaling pathway

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ABSTRACT

Vascular endothelial cell (VEC) dysfunction is associated with the development of coronary heart disease (CHD). Long intergenic non-protein-coding RNA 926 (LINC00926), a kind of long noncoding RNA (lncRNA), has been found to be abnormally expressed in CHD patients. However, the biological role of LINC00926 have not been reported. In our research, we intended to explore the regulatory mechanism of LINC00926 in hypoxia-exposed HUVEC cells (HUVECs). In our *in vitro* study, HUVECs were exposed under hypoxic conditions (5% O_2) for 24 h. RT-qPCR and Western blotting assay were used to detect the mRNA and protein levels. CCK-8 assay, flow cytometry, transwell assay and *in vitro* angiogenesis assay were performed to measure cell proliferation, apoptosis, migration and tube formation, respectively. Bioinformatics analysis was applied to predict the target of LINC00926 and miR-3194-5p, which was verified by dual-luciferase reporter assays. The results showed that LINC00926 was highly expressed in CHD patients and hypoxia-exposed HUVECs. LINC00926 overexpression suppressed cell proliferation, migration and tube formation and increased cell apoptosis. MiR-3194-5p was a target of LINC00926 and can target binding to JAK1 3'UTR. LINC00926 could upregulate JAK1 and p-STAT3 levels *via* miR-3194-5p. In addition, overexpressed LINC00926 suppressed cell proliferation, migration and tube formation and increased cell apoptosis *via* miR-3194-5p/JAK1/STAT3 axis. In summary, LINC00926 aggravated endothelial cell dysfunction *via* miR-3194-5p regulating JAK1/STAT3 signaling pathway in hypoxia-exposed HUVECs.

Key words: CHD; HUVECs; hypoxia; LINC00926; miR-3194-5p; JAK1; STAT3.

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Introduction

Coronary heart disease (CHD) is a common type of cardiovascular diseases (CVDs). In recent years, the incidence of CHD worldwide is increasing, which seriously endangers human life and health.[1] CHD is caused by coronary atherosclerosis, with the gradual narrowing or obstruction of blood vessels due to build-up of atherosclerotic plaque, resulted in myocardial ischemia and hypoxia to induce cardiac dysfunction. It is well known that vascular endothelial cell (VEC) dysfunction plays crucial roles in CVDs, such as atherosclerosis.[2] The correlation between VEC dysfunction and CVD has been widely concerned. Some studies have indicated that VEC dysfunction is characterized by the dysregulation of normal VEC functions, including the vascular tone, the abilities of cell proliferation and migration.[3] Therefore, it is important to explore the molecular mechanism of VEC dysfunction. Long noncoding RNA (lncRNA) is a class of non-coding RNA and its length is more than 200 nucleotides. Numerous studies have shown that lncRNAs are related to the occurrence of CVDs, such as heart failure, atherosclerosis, *etc*.[4,5] Zheng *et al*.[6] showed that knockdown of lncRNATTTY15 regulated miR-186-5p to ameliorate vascular endothelial cell injury induced by hypoxia in CVDs. Guo *et al.*[7] reported that lncRNA-FA2H-2 regulated autophagy and inflammation in atherosclerosis. Besides, Liao *et al*.[8] used high-throughput sequencing to identify differential expression lncRNAs between CHD patients and healthy controls, and found that lncRNA long intergenic non-protein-coding RNA 926 (LINC00926) was highly expressed in the peripheral blood of CHD patients. It suggested that LINC00926 might be related to the development of CHD. At present, the effects of LINC00926 on CHD and VEC function have not been reported. MicroRNA (miRNA) is a class of non-coding single-stranded RNA with a length of about 22 nucleotides. miRNAs are considered to have a regulatory role by targeting to combine with the 3′-UTR of mRNA. Mechanistically, lncRNAs could bind to miRNA to upregulate mRNAs. Huang summarized and emphasized the regulatory network among lncRNA, miRNA and mRNA in CVDs.[9] Predictive analysis of LINC00926 revealed that there might be a binding site between LINC00926 and miR-3194-5p. Until recently, there is little knowledge about miR-3194-5p in CHD and the relationship with LINC00926. Through searching the online database, Janus kinase 1 (JAK1) might be a potential target of miR-3194-5p. In addition, several researches have made clear that signal transducer and activator of transcription 3 (STAT3) is involved in the progress of CVD, including vascular endothelial cell dysfunction.[10,11] Previous studies have showed that JAK1/STAT3 signaling pathway plays important roles to regulate cell functions in the progression of many diseases.[12,13] It is well-documented that the number of human umbilical vein endothelial cells (HUVECs) and their angiogenic capacity, which are closely associated with cardiovascular events and mortality, can be characterized by cell proliferation, migration and tube formation experiment.[14] In this study, HUVECs were used as a model system *in vitro* to investigate the underlying molecular mechanism of LINC00926 in CHD.

Materials and Methods

Clinical samples

Clinical blood sample of thirty CHD patients were collected. Another fifteen healthy subjects for physical examination were selected as control group. None of the healthy subjects had a history of cardiac disease. This study was approved by the Ethics Committee of Jilin Medical University and all participants signed informed consent.

Cell culture

We purchased HUVECs from CTCC (Wuhan, China). RPMI-1640 cell culture medium (Hyclone) was used to culture HUVECs, supplemented with 10% fetal bovine serum (FBS; Gibco) and 100 U/mL streptomycin/penicillin. Cells were put in 37°C, 5%CO_2 incubator. Cells in the hypoxia group were exposed under hypoxic condition (5% O_2) for 24 h.

Cell transfection

Overexpressed LINC00926 vector was constructed by cloning the full length LINC00926 cDNA into pcDNA3.1 vector. The empty plasmid was served as the negative control (NC). LINC00926 si-RNA, miR-3194-5p mimics/mimics NC, miR-3194-5p inhibitor/inhibitor NC were purchased from GENCEFE (Wuxi, China). When cell confluence reached 70~80%, HUVECs were transfected with overexpressed LINC00926 vector, LINC00926 si-RNA, miR-3194-5p mimics/mimics NC or miR-3194-5p inhibitor/inhibitor NC using Lipofectamine 2000 (Invitrogen, Waltham, MA, USA) on the basis of the manufacturer's instructions. After being transfected for 48 h, cells were harvested for subsequent experiments.

RT-qPCR analysis

We extracted total RNA from blood using RNAliquid Blood RNA Kit (Aidlab Bio., Beijing, China). Synthesis of cDNA was completed using First strand cDNA Synthesis Kit. RT-qPCR was conducted with specific primers and SYBR Green qPCR Master Mix. The expression levels of LINC00926 and JAK1 were normalized to GAPDH. The expression levels of miR-3194-5p were normalized to U6. The $2^{-\Delta\Delta CT}$ method was used to analyze the gene expression levels in each group. The primer sequences were listed in Table 1.

Table 1. The primer sequences for RT-qPCR.

Genes	Primer sequences
miR-3194-5p-F	ACACTCCAGCTGGGGGCCAGCCACCAGGA
miR-3194-5p-R	TGGTGTCGTGGAGTCG
JAK1(human)- F	ACGCTCTGGGAAATCTGCTA
JAK1(human) -R	ATGATGGCTCGGAAGAAAGG
LINC00926(human)-F	AGTAGGGACGAGGTTTCAC
LINC00926(human)-R	CAGCCATTTCATCTTCACA
U6-F	CTCGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT
GAPDH (human) -F	AGAAGGCTGGGGCTCATTTG
GAPDH (human) -R	AGGGGCCATCCACAGTCTTC

Western blotting analysis

We extracted total protein from cells using RIPA lysis buffer. Protein quantification was performed using BCA Protein Quantification Kit (Biosharp, Hefei, China) according to the protocol. Sample protein (25 μg) were loaded, followed by SDS-PAGE electrophoresis. Then the purpose tape was transferred to polyvinylidene fluoride (PVDF) membranes. After rinsed with TBST for 5 min (3 times), the membranes were blocked with 5% skim milk for 2 h at 37°C. After washing by TBST for three time, membranes were immersed in the primary antibodies incubation solution (anti-JAK1; dilution 1:1000; Proteintech, Chicago, IL, USA); anti-STAT3 (dilution 1:1000; Proteintech); anti-p-STAT3 (dilution 1:1000; Affinity, Jiangsu, China); anti-GAPDH (dilution 1:5000; Proteintech) overnight at 4°C. The secondary antibodies (goat anti-rabbit or goat anti-mouse HRP-conjugated IgG; dilution 1:5000; ZSGB-BIO, Beijing, China) were added with the membranes at 37°C for 1 h. ECL luminescent solution was added to the membrane, and images were taken using Integrated chemiluminescence instrument. Quantitative analysis was performed using Image J software.

CCK-8 assay

CCK-8 was applied to assess the cell proliferation. HUVECs (1×10^5cells/mL) were cultured in 96-well plates with 37°C, 5%CO_2. Twenty-four hours later, CCK-8 solution (20 μL) was added into each well. After 3 h of incubation under dark conditions, the OD value at 450 nm was detected by enzyme-labeled detector (MK3; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Flow cytometry

Cell apoptosis was detected with Annexin V-FITC Apoptosis Detection Kit (BestBio, Nanjing, China) according to the manufacturer's instructions. Briefly, HUVECs were digested by trypsin solution, then washed by PBS twice. HUVECs were re-suspended using 1×Binding Buffer (300 μL) and adjusted to 1×10^6 cells/mL. Under the dark conditions, cells were incubated at 2-8°C with Annexin V-FITC (5 μL) for 15 min and PI (10 μL) for 5 min, respectively. Flow cytometry analysis were performed using a Flow cytometer (BD-FACSVerse, BD Biosciences, Franklin Lakes, NJ, USA). The green fluorescence intensity of FITC was detected at excitation/emission wavelengths of 488/525 nm in FL1 channel. The red fluorescence intensity of PI was detected at excitation/emission wavelengths of 535/615 nm in FL2 channel. Finally, the data were analyzed by FlowJo 7.6 software.

Transwell

We applied transwell to assess cell migration. The cell suspensions of HUVECs were put in the upper chamber of Transwell and incubated in serum-free medium. We added 600 μL medium containing serum into the lower chamber. The transwell chamber was put in 37°C, 5% CO_2. After 48 h, the liquid in the upper chamber was moved away. Then we used PBS to wash cells twice. Paraformaldehyde (4%) was added to fix cells for 30 min. Crystal violet (0.1%) was also added to stain cells for 10 min. The polycarbonate ester film was carefully cut off from the base of the upper chamber and photographed under a microscope.

In vitro angiogenesis assay

Matrigel matrix were added into 24-well plates, solidified at 37°C for 30 min. Subsequently, cell suspensions of HUVECs (2×10^5cell/mL) were added into each well. After 6 h of incubation in 37°C, 5% CO_2, HUVECs were stained with Calcein AM (0.25μg/mL) for 30 min, then observed under a fluorescence microscope (BZ-8000; Keyence, Osaka, Japan) at the excitation/emission wavelengths with 495/515 nm.

Dual luciferase reporter assay (LRA)

The wild-type (WT) or mutant type (Mut) of LINC00926 or 3' untranslated regions (UTR) of JAK1 was inserted into psiCHECK2 vector. HEK293T cells were co- transfected with 50 ng reporter vectors and 20 μM miR-3194-5p mimics/mimics NC using Lipofectamine 2000 on the basis of the instructions. After 48 hours of transfection, LRA System (Promega, Beijing, China) was performed to detect the luciferase activity.

Statistical analysis

Each experiment was repeated three times. GraphPad Prism 7.0 was used for statistical analysis. All data were expressed by mean value ± SD). The differences among two or more groups were analyzed using the unpaired Student's *t*-test or ANOVA. A value of $p<0.05$ was considered as statistically significant.

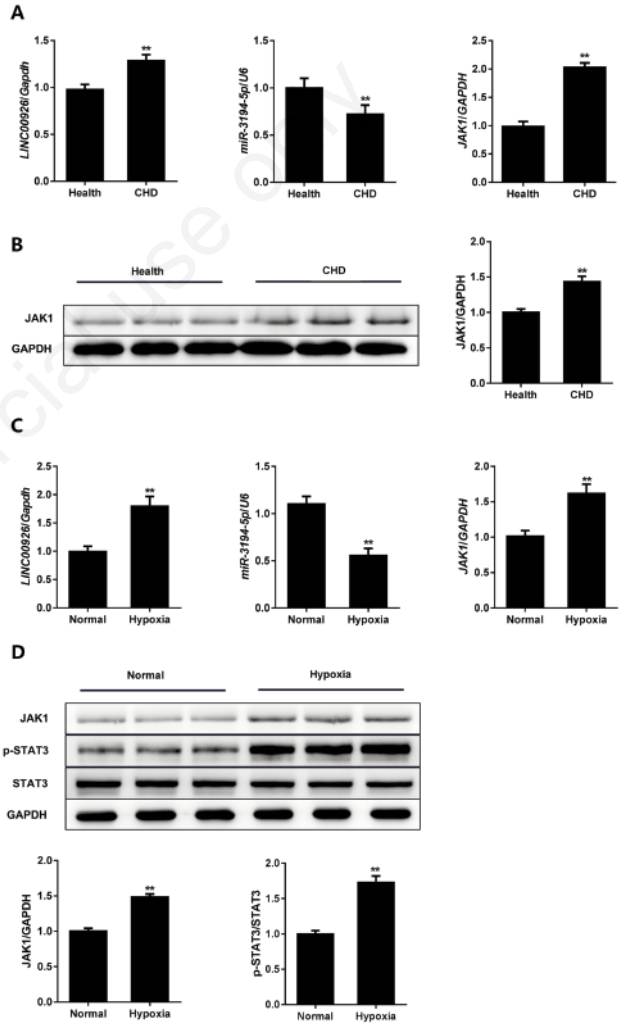

Figure 1. The expression levels of LINC00926, miR-3194-5p, JAK1 and STAT3 in CHD patients and hypoxia-exposed HUVECs. A) RT-qPCR was performed to analyze the mRNA expressions of LINC00926, miR-3194-5p, JAK1 in CHD patients. B) Western blot was used to detect the protein expression of JAK1 in CHD patients; **p<0.01 *vs* health. C) RT-qPCR was performed to analyze the mRNA expressions of LINC00926, miR-3194-5p, JAK1 in HUVECs. D) Western blot was applied to detect the protein levels of JAK1, STAT3, p-STAT3 in HUVECs; **p<0.01 *vs* normal.

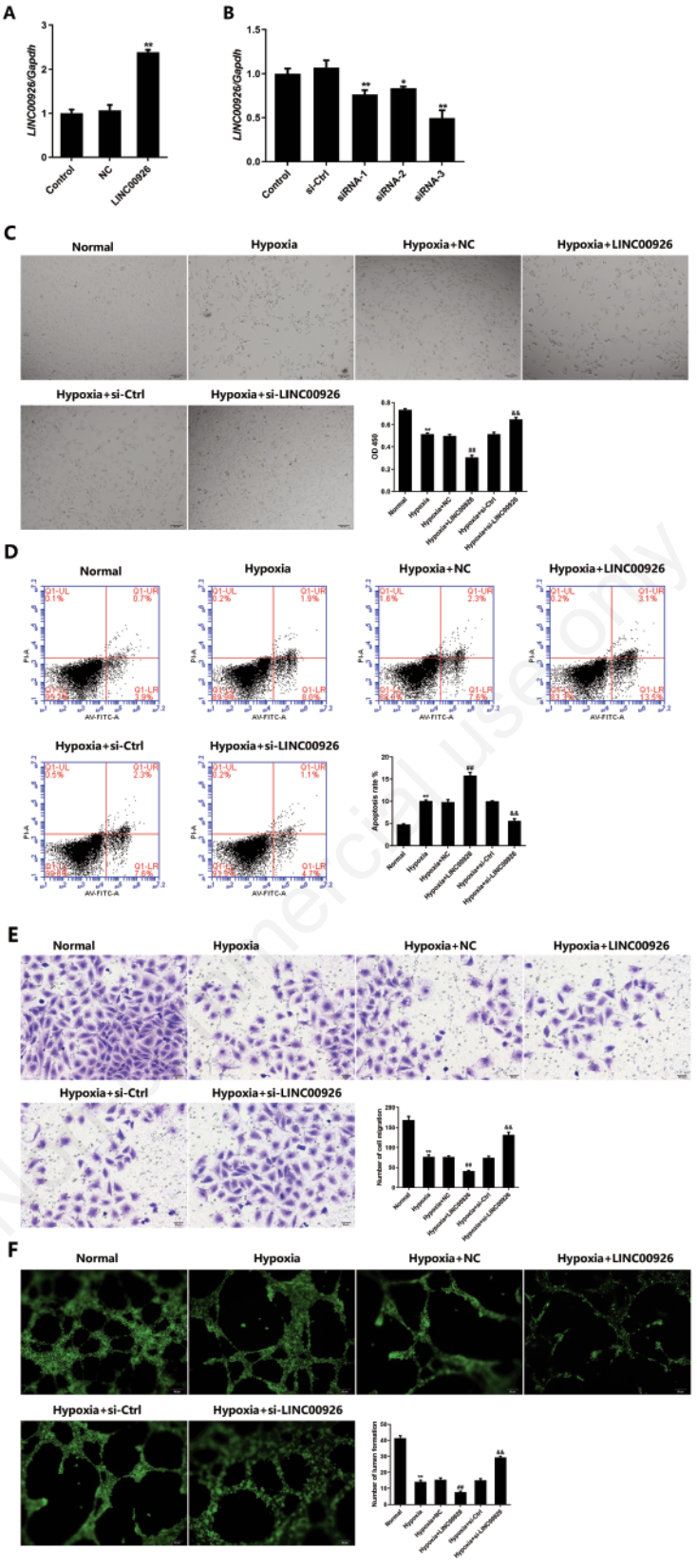

Figure 2. Effects of LINC00926 on HUVECs. A) HUVECs were transfected with overexpressed LINC00926 plasmid. RT-qPCR was applied to detect the overexpression efficiency; **p<0.01 *vs* control. B)Three interference sequences of LINC00926 were transfected to HUVECs. RT-qPCR was applied to detect the interference efficiency; *p<0.05; **p<0.01 *vs* control. Hypoxia-induced HUVECs were transfected with overexpressed LINC00926 plasmid or LINC00926 siRNA. CCK-8 assay (C), flow cytometry (D), Transwell assay (E), *in vitro* angiogenesis assay (F) was conducted to evaluate cell proliferation, apoptosis, migration and tube formation, respectively; **p<0.01 *vs* normal; ##p<0.01 *vs* hypoxia+NC; &&p<0.01 *vs* hypoxia+si-control.

Results

LINC00926 and JAK1 were upregulated, while miR-3194-5p was downregulated in CHD patients and hypoxia-exposed HUVECs.

As shown in Figure 1A, compared to the health group, the LINC00926 and JAK1 expression were both significantly increased in CHD, while miR-3194-5p was significantly decreased. Consistent with gene expression, the JAK1 protein expression was also notably increased in CHD group (Figure 1B).

On the other side, the levels of LINC00926, miR-3194-5p, JAK1, STAT3 were also detected in hypoxia-exposed HUVECs. Compared to the normal group, the mRNA expression of LINC00926 and JAK1 were significantly increased, while miR-3194-5p was significantly decreased in hypoxia group (Figure 1C). Compared to normal group, the JAK1 protein expression and p-STAT3/STAT3 were obviously increased (Figure 1D). These results indicated that LINC00926, miR-3194-5p, JAK1 and STAT3 might be involved in the occurrence of CHD.

Overexpressed LINC00926 reduced cell proliferation, migration and tube formation, but facilitated cell apoptosis in hypoxia-exposed HUVECs

To investigate the role of LINC00926 in hypoxia-exposed HUVECs, HUVECs were transfected with overexpressed LINC00926 plasmid or LINC00926 siRNA, that were cultured under hypoxic conditions. First, the results of figure 2A showed that the expression of LINC00926 was evidently increased after transfection with overexpressed LINC00926 plasmid. We designed and synthesized three interference sequences of LINC00926. The level of LINC00926 was significantly decreased after transfection with siRNA-LINC00926 (Figure 2B). The siRNA-3 with the highest silencing efficiency, was used for subsequent experiments. We used CCK-8 assay, flow cytometry, transwell assay, *in vitro* angiogenesis assay to evaluate the abilities of cell proliferation, apoptosis, migration and tube formation, respectively. Compared to the normal group, hypoxia treatment reduced the abilities of cell proliferation, migration and tube formation, which were further decreased after transfection with LINC00926 overexpression (Figure 2 C,E,F). On the other side, si-LINC00926 markedly improved these abilities. Flow cytometry analysis revealed that hypoxia treatment induced cell apoptosis compared with the normal group. LINC00926 overexpression further promoted cell apoptosis, whereas si-LINC00926 obviously inhibited cell apoptosis (Figure 2D). Together, these findings confirmed that LINC00926 overexpression aggravated cell dysfunction in hypoxia-exposed HUVECs.

LINC00926 overexpression downregulated the miR-3194-5p expression, but upregulated the JAK1 and p-STAT3 expression in hypoxia-exposed HUVECs

According to the figure 3A, it was found that compared to the normal group, the level of miR-3194-5p was declined in the hypoxia group. LINC00926 overexpression further downregulated

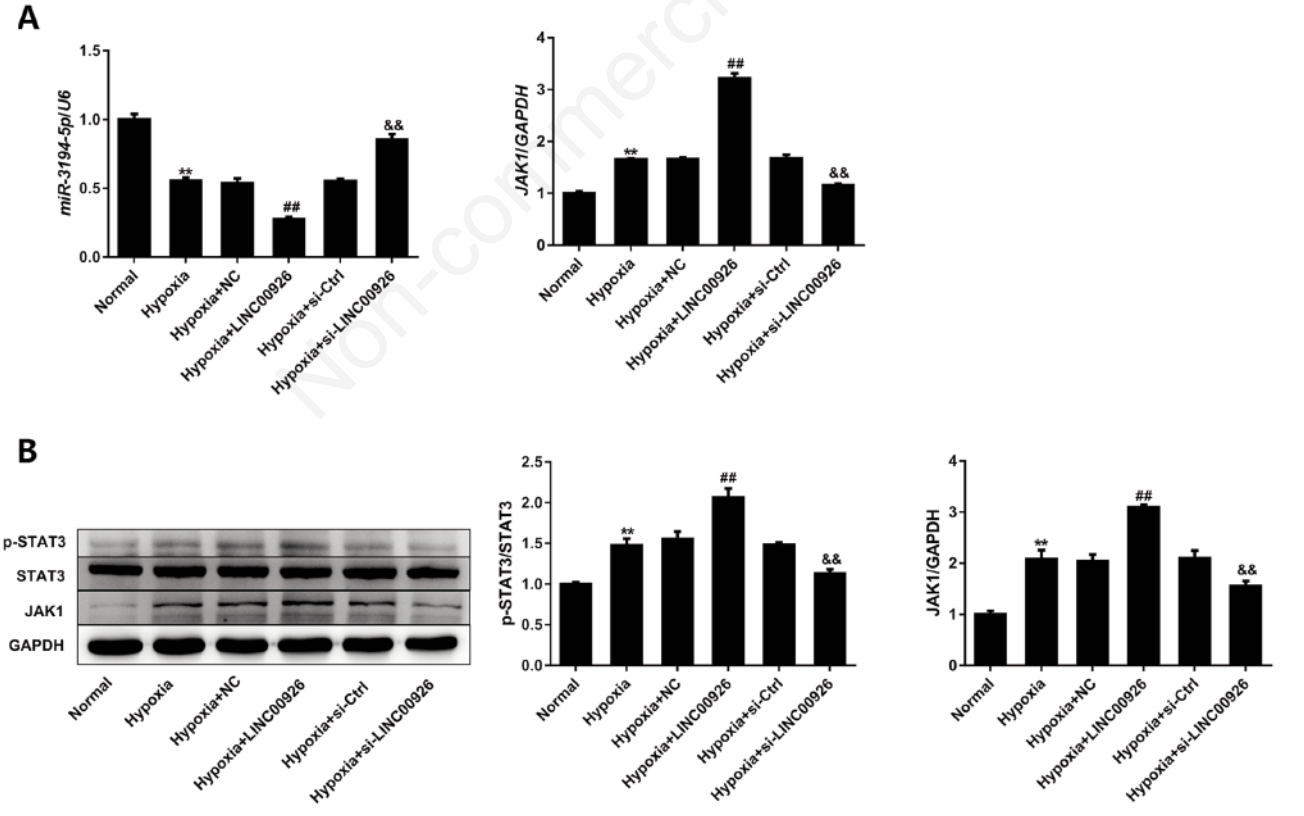

Figure 3. LINC00926 downregulated the level of miR-3194-5p, but upregulated the levels of JAK1 and p-STAT3 in hypoxia-exposed HUVECs. HUVECs were transfected with LINC00926 overexpression or si-LINC00926, followed by treating with hypoxia. A) RT-qPCR was performed to analyze the mRNA expressions of miR-3194-5p and JAK1. B) Western blot was used to detect the protein expression of JAK1, STAT3, p-STAT3; **p<0.01 *vs* normal; $^{\#\#}$p<0.01 *vs* hypoxia+NC; $^{\&\&}$p<0.01 *vs* hypoxia+si-control.

the miR-3194-5p expression, while si-LINC00926 upregulated the miR-3194-5p expression. On the contrary, the mRNA level of JAK1 was increased under hypoxia treatment. LINC00926 overexpression further upregulated the JAK1 expression, while si-LINC00926 downregulated the JAK1 expression. Western blot results showed that the p-STAT3/STAT3 ratio and the protein expression of JAK1 were increased in the hypoxia group, that were further improved after LINC00926 overexpression. However, si-LINC00926 partially reversed these results (Figure 3B). The above data suggested that overexpressed LINC00926 could downregulate the miR-3194-5p expression, leading to upregulation of JAK1 expression and STAT3 phosphorylation in hypoxia-exposed HUVECs.

LINC00926 regulated JAK1/STAT3 signaling pathway via miR-3194-5p

Through Starbase (https://starbase.sysu.edu.cn/index.php) online database, we found that there was a binding site between LINC00926 and miR-3194-5p. The luciferase activity of cells co-transfected with miR-3194-5p mimics and LINC00926-WT was significantly reduced than that of cells co-transfected with mimics NC and LINC00926-WT. And there was no evident change about the luciferase activity in cells co-transfected with miR-3194-5p mimics or mimics NC and LINC00926-MUT (Figure 4A). The target protein of miR-3194-5p was predicted by searching TargetScan online database (http://www.targetscan.org/), showing that miR-3194-5p may bind to JAK1 3'UTR. And the results of figure 4B have confirmed the binding between miR-3194-5p and the 3'UTR of JAK1.

Next, hypoxia-exposed HUVECs were transfected with miR-3194-5p mimics/ mimics NC or miR-3194-5p inhibitor/inhibitor NC. We found that the mRNA and protein expression of JAK1 were markedly decreased after transfected with miR-3194-5p mimics, while that were obviously increased after transfected with miR-3194-5p inhibitor (Figure 4C). At the same time, compared with mimic/inhibitor NC, the p-STAT3/STAT3 ratio was decreased distinctly in the miR-3194-5p mimics group, but that was significantly increased in the miR-3194-5p inhibitor group (Figure 4D). Together, these data prompted that LINC00926 could regulate JAK1/STAT3 signaling *via* miR-3194-5p in HUVECs exposed to hypoxia.

Overexpressed LINC00926 induced cell dysfunction *via* miR-3194-5p regulating JAK1/STAT3 signaling pathway

To explore whether LINC00926 affects the cell function of HUVECs *via* miR-3194-5p/JAK1/STAT3 axis HUVECs were co-transfected with overexpressed LINC00926 plasmid and miR-

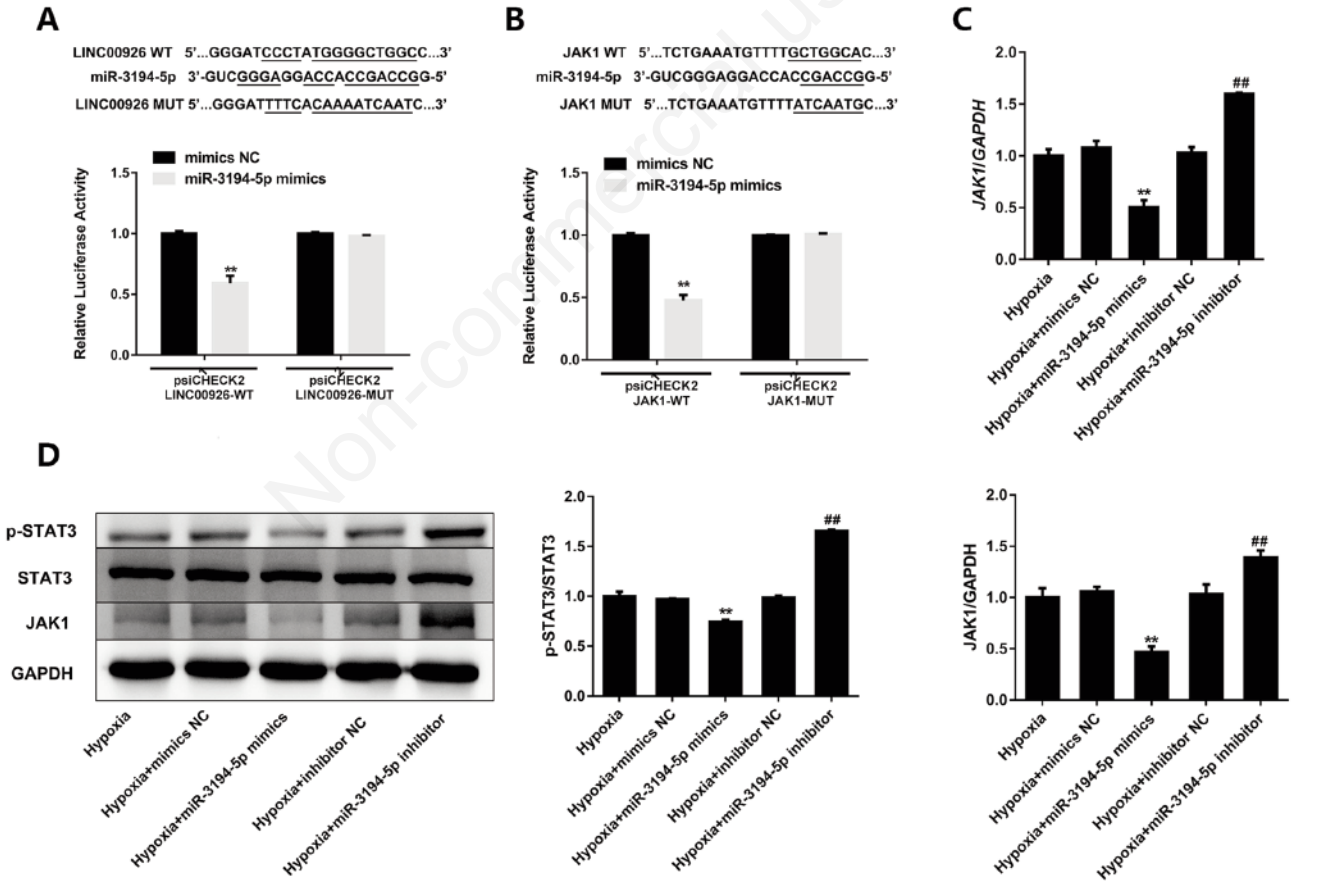

Figure 4. LINC00926 regulated JAK1/STAT3 signaling pathway *via* miR-3194-5p. Luciferase reporter vectors and miR-3194-5p mimics/mimics NC were co-transfected to HUVECs. A) LRA was applied to confirm the interactive relationship between LINC00926 and miR-3194-5p. B) The binding site between miR-3194-5p and JAK1; **p<0.01 *vs* mimics NC. C) HUVECs were transfected with miR-3194-5p mimic/inhibitor. JAK1 expression was analyzed by RT-qPCR. D) The protein expressions of JAK1, STAT3, p-STAT3 were examined by Western blot; **p<0.01 *vs* hypoxia+mimics NC; ##p<0.01 *vs* hypoxia+inhibitor NC.

3194-5p mimics/mimics NC, followed by treating with hypoxia. As shown in Figure 5 A,B, LINC00926 overexpression significantly upregulated the level of JAK1, whereas miR-3194-5p mimics reversed the effect of LINC00926 overexpression. Meanwhile, LINC00926 overexpression increased the ratio of p-STAT3/STAT3, which was also reversed by miR-3194-5p mimics. Furthermore, we found that LINC00926 overexpression obviously suppressed cell proliferation, migration and tube formation. More importantly, these effects were positively rescued by miR-3194-5p mimics (Figure 5 C,E,F). On the contrary, miR-3194-5p mimics reversed the stimulative effects of LINC00926 overexpression on HUVECs cell apoptosis (Figure 5D). Therefore, rescue experiments demonstrated that overexpressed LINC00926 induced cell dysfunction through miR-3194-5p regulating JAK1/STAT3 signaling pathway in HUVECs exposed to hypoxia.

Discussion

CHD involves the reduction of blood flow to the heart muscle due to build-up of atherosclerotic plaque in the arteries of the heart. When the plaque ruptures or its surface is eroded, the formation of thrombosis may aggravate vascular lumen stenosis, or even acute occlusion. Beclin1-induced VEC dysfunction can promote thrombosis after plaque rupture.[15] Clinicopathological examination showed that there are serious structural and functional damage in endothelial cells in patients with CVD.[16] In addition, accumulating evidence indicates that lncRNAs regulates VEC function. For instance, Hosen *et al*.[17] indicated that lncRNA PUNISHER was an important regulator on angiogenic response and endothelial cells function *via* NFkB in CHD. Liu *et al*.[18] showed that ANRIL regulated cell dysfunction of HUVECs by inhibiting let-7b targeting the TGF-βR1/Smad pathway. Existing literature reported that LINC00926 was significantly expressed in patients with CHD in comparison to healthy participants.[8] Nevertheless, the effects of LINC00926 in CHD or VECs have not been documented. In the present study, LINC00926 was upregulated in CHD patients compared to health participants, that is consistent with the existing literature. Our findings showed that LINC00926 expression was increased in HUVECs during exposure to hypoxia. Hypoxia inhib-

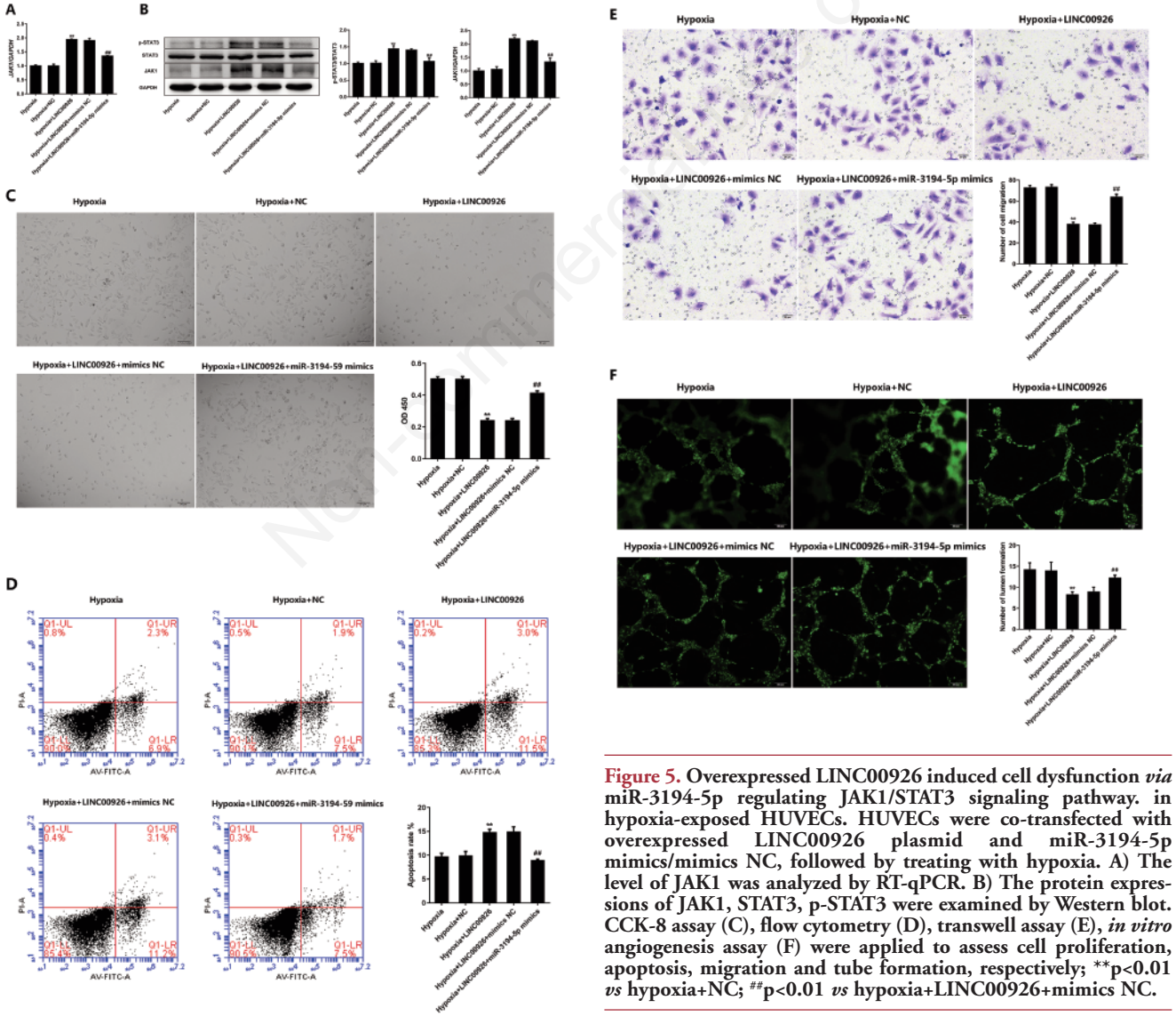

Figure 5. Overexpressed LINC00926 induced cell dysfunction *via* miR-3194-5p regulating JAK1/STAT3 signaling pathway. in hypoxia-exposed HUVECs. HUVECs were co-transfected with overexpressed LINC00926 plasmid and miR-3194-5p mimics/mimics NC, followed by treating with hypoxia. A) The level of JAK1 was analyzed by RT-qPCR. B) The protein expressions of JAK1, STAT3, p-STAT3 were examined by Western blot. CCK-8 assay (C), flow cytometry (D), transwell assay (E), *in vitro* angiogenesis assay (F) were applied to assess cell proliferation, apoptosis, migration and tube formation, respectively; **p<0.01 *vs* hypoxia+NC; ##p<0.01 *vs* hypoxia+LINC00926+mimics NC.

ited cell proliferation, migration and tube formation and induced cell apoptosis in HUVECs, that suggesting that hypoxia can induce HUVEC dysfunction. Next, the effects of LINC00926 on HUVECs were determined. LINC00926 upregulation reduced cell proliferation, migration and tube formation, but facilitated cell apoptosis in hypoxia-exposed HUVECs. Hence, we noted that LINC00926 overexpression could induce cell dysfunction in hypoxia-exposed HUVECs.

Increasing evidence confirm that numerous lncRNAs function as competing endogenous RNA through interacting with miRNA to regulate mRNA expressions. The interactive relationship between miRNAs and mRNAs has also been experimentally identified. LncRNAs, miRNAs and mRNAs were involved in constructing miRNA-lncRNA-mRNA interaction network, which function critically in CVD.[19] Lin *et al*.[20] reported that lncRNA THRIL relieved myocardial injury *via* miRNA-424/TXNIP/p53 aixs in CHD mice. Huang *et al*.[21] confirmed that *lnc-SLC15A1-1* upregulated CXCL10 and CXCL8 levels by sponging miRNA in HUVECs. In our study, both hypoxia treatment and overexpressed LINC00926 downregulated the level of miR-3194-5p, upregulated the levels of JAK1 and p-STAT3. Meanwhile, silencing LINC00926 reversed these effects. Furthermore, the results of bioinformatics analysis and LRA determined that LINC00926 interacted with miR-3194-5p, leading to upregulation of JAK1 level and STAT3 phosphorylation. In general, LINC00926 acts as a sponger of miR-3194-5p to regulate JAK1/STAT3 signaling pathway in hypoxia-exposed HUVECs.

STAT3, a member of the family of STATs protein, has been proved the impact on various kinds of cell processes, including cell growth, apoptosis, migration and cell death.[22,23] Also, it is well documented that JAK mediates STAT3 phosphorylation in many cell types.[24,25] Chen *et al*.[10] have reported that STAT3 phosphorylation have a vital role in endothelial cell dysfunction during the development of atherosclerosis. In this study, rescue experiments were performed to investigate the mechanisms of LINC00926 *via* miR-3194-5p/JAK1/STAT3 axis regulating cellular functions in hypoxia-exposed HUVECs. Our data showed that overexpressed LINC00926 upregulated the levels of JAK1 and p-STAT3, while miR-3194-5p downregulated these levels. Meanwhile, LINC00926 overexpression inhibited cell proliferation, migration and tube formation and promoted cell apoptosis. Notably, these alterations were reversed by miR-3194-5p mimics. Thus, these findings demonstrated that upregulation of LINC00926 induced cell dysfunction via miR-3194-5p/JAK1/STAT3 axis in hypoxia-exposed HUVECs.

In conclusion, our data in this study reveals that LINC00926 aggravate endothelial cell dysfunction by sponging miR-3194-5p and regulating JAK1/STAT3 signaling pathway in hypoxia-exposed HUVECs. This study demonstrates that LINC00926 plays an essential role in the occurrence of CHD, and it provides a potential diagnostic biomarker for CHD treatment.

References

1. Xu H, Zhang X, Yu K, Zhang G, Yfei Shi Y, Jiang Y. Analysis on the expression and prognostic value of LncRNA FAF in Patients with coronary heart disease. Biomed Res Int 2020;2020:9471329.
2. Gimbrone MA Jr, García-Cardeña G. Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res 2016;118:620-36.
3. Chamorro-Jorganes A, Araldi E, Suárez Y. MicroRNAs as pharmacological targets in endothelial cell function and dysfunction. Pharmacol Res 2013;75:15-27.
4. Archer K, Broskova Z, Bayoumi AS, Teoh JP, Davila A, Tang Y, et al. Long non-coding RNAs as master regulators in cardiovascular diseases. Int J Mol Sci 2015;16:23651-67.
5. Li H, Zhu H, Ge J. Long noncoding RNA: Recent updates in atherosclerosis. Int J Mol Sci 2016;12:898-910.
6. Zheng J, Zhuo YY, Zhang C, G-Y Tang, Gu X-Y, Wang F. LncRNA TTTY15 regulates hypoxia-induced vascular endothelial cell injury via targeting miR-186-5p in cardiovascular disease. Eur Rev Med Pharmacol Sci 2020;24:3293-301.
7. Guo FX, Wu Q, Li P, Zheng L, Ye S, Daiet X-Y, al. The role of the LncRNA-FA2H-2-MLKL pathway in atherosclerosis by regulation of autophagy flux and inflammation through mTOR-dependent signaling. Cell Death Differ 2019;26:1670-87.
8. Liao J, Wang J, Liu Y, Li J, Duan L. Transcriptome sequencing of lncRNA, miRNA, mRNA and interaction network constructing in coronary heart disease. BMC Med Genomics 2019;12:124.
9. Huang Y. The novel regulatory role of lncRNA-miRNA-mRNA axis in cardiovascular diseases. J Cell Mol Med 2018;22:5768-75.
10. Chen Q, Lv J, Yang W, Xu B, Wang Z, Yu Z, et al. Targeted inhibition of STAT3 as a potential treatment strategy for atherosclerosis. Theranostics 2019;9:6424-62.
11. Hu Y, Xu R, He Y, Zhao Z, Mao X, Linet L, et al. Downregulation of microRNA−106a−5p alleviates ox−LDL−mediated endothelial cell injury by targeting STAT3. Mol Med Rep 2020;22:783-91.
12. Bonnin DA, Havrda MC, Lee MC, Liu H, Zhang Z, Nguyen LN, et al. Secretion-mediated STAT3 activation promotes self-renewal of glioma stem-like cells during hypoxia. Oncogene 2018;37:1107-18.
13. Kurdi M, Sivakumaran V, Duhé RJ, Aon MA, Paolocci N, Booz GW. Depletion of cellular glutathione modulates LIF-induced JAK1-STAT3 signaling in cardiac myocytes. Int J Biochem Cell Biol 2012;44:2106-15.
14. Zhai S, Zhang XF, Lu F, Chen WG, He X, Zhanget C-F, al. Chinese medicine GeGen-DanShen extract protects from myocardial ischemic injury through promoting angiogenesis via up-regulation of VEGF/VEGFR2 signaling pathway. J Ethnopharmacol 2021;267:113475.
15. Yu F, Zhang Y, Wang Z, Gong W, Zhang C. et al. Hsa_circ_0030042 regulates abnormal autophagy and protects atherosclerotic plaque stability by targeting eIF4A3. Theranostics 2021;11:5404-17.
16. Zhang Q, Liu J, Duan H, Li R, Peng W, Wu C. Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. J Adv Res 2021;34:43-63.
17. Hosen MR, Nickenig G, Jansen F. Coronary artery disease ameliorates extracellular vesicle lncRNA PUNISHER regulates angiogenic response andendothelial cells function via NFkB-dependent mechanism. Eur Heart J 2022;43: ehac544.2935.
18. Liu X, Li S, Yang Y, Sun Y, Yang Q, Gu N, et al. The lncRNA ANRIL regulates endothelial dysfunction by targeting the let-7b/TGF-βR1 signalling pathway. J Cell Physiol 2021;236: 2058-69.
19. Mao J, Zhou Y, Lu L, Zhang P, Ren R, Wang Y, et al. Identifying a Serum exosomal-associated lncRNA/circRNA-miRNA-mRNA network in coronary heart disease. Cardiol Res Pract 2021;2021:6682183.
20. Lin L, Bao J. Long non-coding RNA THRIL is upregulated in coronary heart disease and binds to microRNA-424 to upregu-

late TXNIP in mice. Microvasc Res 2021;138:104215.
21. Huang YC, Tsai TC, Chang CH, Chang K-T, Ko P-H, Lai L-C. Indoxyl Sulfate elevated Lnc-SLC15A1-1 upregulating CXCL10/CXCL8 expression in high-glucose endothelial cells by sponging microRNAs. Toxins (Basel) 2021;13:873.
22. Kim M, Morales LD, Jang IS, Cho Y-Y, Kim DJ. Protein tyrosine phosphatases as potential regulators of STAT3 signaling. Int J Mol Sci 2018;19:2708.
23. Bromberg JF. Activation of STAT proteins and growth control. Bioessays 2001;23:161-9.
24. Murray PJ. The JAK-STAT signaling pathway: input and output integration. J Immunol 2007;178:2623-9.
25. Ni CW, Hsieh HJ, Chao YJ, Wang DL. Interleukin-6-induced JAK2/STAT3 signaling pathway in endothelial cells is suppressed by hemodynamic flow. Am J Physiol Cell Physiol 2004;287:C771-80.

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