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MiR-593-5p promotes the development of hypoxic-induced pulmonary hypertension via targeting PLK1

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Abstract. – OBJECTIVE: The aim of this study was to investigate the role of microRNA-593-5p (miR-593-5p) in hypoxia-induced pulmonary hypertension (HPH), and to explore its underlying mechanism.

MATERIALS AND METHODS: Sprague-Dawley (SD) rats were housed in a hypoxia environment 8 hours per day for consecutive 4 weeks. After the establishment of the HPH rat model, we detected the right ventricular systolic pressure (RVSP) and right heart hypertrophy index (RVHI) in HPH rats and controls. The expression levels of miR-593-5p and polo-like kinase 1 (PLK1) in rat lungs were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Subsequently, miR-593-5p mimics and inhibitor were constructed and transfected into cells. The proliferation and migration of pulmonary arterial smooth muscle cells (PASMCs) were accessed by cell counting kit-8 (CCK-8) assay and wound healing assay, respectively. The protein level of PLK1 in PASMCs after transfection with miR-593-5p mimics or inhibitor was detected by Western blot. Dual-luciferase reporter gene assay was conducted to verify the binding condition of miR-593-5p and PLK1. Finally, rescue experiments were performed to explore whether the regulatory effect of miR-593-5p on HPH development was associated with PLK1.

RESULTS: RVSP and RVHI in rats of the hypoxic group were significantly higher than those of controls. MiR-593-5p was significantly downregulated while PLK1 was remarkably upregulated in lung tissues of HPH rats than those of controls. Similarly, miR-593-5p expression in PASMCs decreased gradually with the prolongation of hypoxia induction. Overexpression of miR-593-5p remarkably inhibited the proliferation and migration of PASMCs. Subsequently, dual-luciferase reporter gene verified the binding condition of miR-593-5p and PLK1. Both the mRNA and protein levels of PLK1 were negatively regulated by miR-593-5p. Also, rescue experiments demonstrated that the inhibitory effects of miR-593-5p on the proliferation and migration of PASMCs could be reversed by PLK1 overexpression.

CONCLUSIONS: MiR-593-5p is lowly expressed in lung tissues of HPH rats. Meanwhile, it stimulates the proliferation and migration of PASMCs via targeting PLK1, thereby promoting HPH development.

Key Words
Hypoxia-induced pulmonary hypertension (HPH), MicroRNA-593-5p (MiR-593-5p), PLK1, Proliferation.

Introduction

Pulmonary arterial hypertension (PAH) is characterized by a persistent increase in pulmonary vascular resistance caused by heterogeneous diseases. Hyper-proliferation or over-migration of angiogenic cells, such as pulmonary arterial smooth muscle cells (PASMCs), and pulmonary artery endothelial cells (PAECs), are the main causes of PAH[1]. Hypoxia-induced pulmonary hypertension (HPH) resulted from long-term hypoxia. Meanwhile, it is characterized by progressive pulmonary vascular resistance, pulmonary vascular stenosis, and pulmonary vascular remodeling. More seriously, HPH may eventually develop into right heart failure or even death. Previous studies have found that hypoxia-induced pulmonary vasoconstriction and pulmonary vascular remodeling are the major pathogenic factors for HPH. Also, it is considered that hyper-proliferation of PASMCs is of great significance for the occurrence and development of HPH[2].

Currently, multiple influencing factors are involved in the development of HPH. Numerous studies[3-5] have shown that mitogenic factors, apoptosis, transforming growth factors, and corresponding receptors are closely associated with HPH. Also, abnormal expression levels of phosphatase and tensin homolog deleted on chromosome ten (PTEN), bone morphogenetic protein

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type II receptor, and calmodulin can promote the occurrence and progression of HPH[6-8]. Although many efforts have been made in exploring the potential mechanism of HPH, further research on effective treatment of HPH is still needed to improve the clinical outcomes of HPH patients.

MicroRNAs (miRNAs) are a class of non-coding, small RNAs with about 18-25 nucleotides in length. MiRNAs identify specific target miRNAs at the transcriptional level, thereafter regulating multiple biological functions[9,10]. Accumulating evidence has showed the close relationship between miRNAs and HPH[11]. It has been reported that a great number of miRNAs are differentially expressed under hypoxic conditions, such as miR-190, miR-145, miR-103/107, miR-223, and miR-214[12]. For example, miR-103/107 and miR-214 alleviate hypoxia-induced vascular remodeling of PASMCs by targeting HIF-1α and CCNL2, respectively[13]. Moreover, miRNA let-7g inhibits hypoxia-induced proliferation and cell cycle arrest of PASMCs by targeting c-myc[14]. However, the exact role of miR-593-5p in HPH remain unclear yet.

Materials and Methods

Construction of the HPH Rat Model

Totally 16 male Sprague-Dawley (SD) rats weighing 180-220 g were randomly assigned into two groups, with 8 in each group. Rats in the control group were maintained in an environment with 21% O_2. However, rats in the hypoxic group were housed in a low-oxygen chamber with ordinary pressure (10±0.5% O_2) for 8 hours every day and transferred to a normal environment with 21% O_2. The HPH model was successfully established in rats after induction with hypoxia for consecutive 4 weeks. This study was approved by the Ethics Committee of Capital Medical University.

Determination of RVSP and RVHI in Rats

Rats were intraperitoneally injected with 50 mg/kg pentobarbital sodium for anesthesia. A tracheotomy was performed by mechanical ventilation (8 ml/kg of tidal volume and 80 beats/min of respiratory rate), and the right ventricular systolic pressure (RVSP) and right heart hypertrophy index (RVHI) were detected. Subsequently, the rats were sacrificed, and the right ventricular free wall was isolated. The weight of the right ventricular free wall (RV) and left ventricle + ventricular septum (LV+S) was recorded. RVHI=RV/(LV+S).

Cell Culture and Anoxia Treatment

PASMCs were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS, Gibco, Rockville, MD, USA), 1% penicillin and streptomycin. Based on different anoxia treatments, PASMCs were divided into three groups, including: the control group (21% O_2, 5% CO_2, 37°C), the 24-h hypoxic group (3% O_2, 5% CO_2, 37°C), and the 48-h hypoxic group (3% O_2, 5% CO_2, 37°C).

Cell Transfection

PASMCs were seeded into 24-well plates one day prior to cell transfection. Briefly, 20 pmol transfection plasmid or 1 μL Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) was diluted into 50 μL serum-free DMEM, respectively. Then, the two solutions were mixed together and maintained at room temperature for 15 min. Subsequently, PASMCs were incubated with the mixture for 4-6 h, followed by replacement of serum-containing DMEM.

Cell Counting Kit-8 (CCK-8) Assay

PASMCs were seeded into 6-well plates after corresponding treatments. 10 μL CCK-8 reagent (Dojindo, Kumamoto, Japan) was added in each well, followed by incubation at 37°C for 2 hours in the dark. Optical density (OD) value of each sample at the wavelength of 450 nm was detected by a microplate reader (Bio-Rad, Hercules, CA, USA).

Wound Healing Assay

PASMCs were seeded into 12-well plates for overnight culture. When the confluence was up to 80%, a sterile 10 μL micro-pipette tip was used to scratch the cell plate vertically. After removing exfoliated cells with phosphate-buffered saline (PBS; Beyotime, Shanghai, China), serum-free medium was replaced for 48-h-incubation. Cell migration was observed under an inverted microscope (Nikon, Tokyo, Japan). The width of the scratch was measured and photographed using Image-Pro Plus 6.0 software (Silver Springs, MD, USA).

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was extracted from the tissues and cells using the TRIzol kit (Invitrogen, Carlsbad, CA, USA), respectively, followed by measure-

ment of RNA concentration using an ultraviolet spectrophotometer (Hitachi, Tokyo, Japan). The complementary Deoxyribose Nucleic Acid (cDNA) was synthesized according to the instructions of the PrimeScriptTM RT MasterMix kit (Invitrogen, Carlsbad, CA, USA). QRT-PCR reaction conditions were as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, for a total of 40 cycles. The relative expression level of the target gene was expressed by $2^{-\Delta\Delta Ct}$. Primers used in this study were: Polo-like kinase 1 (PLK1), F: 5'-ATGCGGAGCCAGCACCTTAA-3', R: 5'-TCCCTTGCATGTAATAGGG-3'; GAPDH, F: 5'-ACCCACTCCTCCACCTTTGA-3', R: 5'-CTGTTGCTGTAGCCAAATTCGT-3'; MicroRNA-593-5p,F:5'-GTGGTCATGTAGCGTCGATTT-3', R: 5'-ATTTGCATGCTACGCTATTAC-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'.

Western Blot

PASMCs were lysed for protein extraction. The concentration of each protein sample was determined by the bicinchoninic acid (BCA) kit (Abcam, Cambridge, MA, USA). Extracted protein was separated by gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After incubation with primary and secondary antibodies (Cell Signaling Technology, Danvers, MA, USA), immune-reactive bands were exposed by enhanced chemiluminescence (ECL) method (Thermo Fisher Scientific, Waltham, MA, USA).

Dual-Luciferase Reporter Gene Assay

Cells were first seeded into 12-well plates, and co-transfection was performed when the confluence was up to 70%. Three replicates were set for each sample. Wild-type and mutant-type PLK1 sequences were constructed. Briefly, cells were co-transfected with 50 pmol/L miR-593-5p mimics or negative control and 80 ng wild-type or mutant-type PLK1 for 48 h, respectively. Luciferase activity was detected using the Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 Software (IBM, Armonk, NY, USA) was used for all statistical analysis. Quantitative data were represented as mean ± standard deviation ($\bar{x}\pm s$). The *t*-test was used to compare the differences between the two groups. $p<0.05$ was considered statistically significant.

Results

MiR-593-5p Was Lowly Expressed in Lung Tissues of HPH Rats

We found that RVSP and RVHI in rats of the hypoxic group were remarkably higher than those of the control group, indicating the successful establishment of the HPH rat model (Figure 1A and 1B). Subsequently, qRT-PCR was performed to detect the mRNA expression levels of miR-593-5p and PLK1 in lung tissues of rats. Results showed that compared with controls, the expression of miR-593-5p in lung tissues of HPH rats was significantly down-regulated, whereas PLK1 was significantly up-regulated (Figure 1C and 1D). This suggested that miR-593-5p and PLK1 participated in the occurrence and progression of HPH.

MiR-593-5p Inhibited the Proliferation and Migration of PASMCs

PASMCs underwent hypoxia induction for 24 h and 48 h, respectively. QRT-PCR results found that the expression level of miR-593-5p in the 24 h-hypoxic group and the 48-h hypoxic group was remarkably lower than that of the control group (Figure 2A). To further explore the biological function of miR-593-5p, miR-593-5p mimics and inhibitor were constructed and transfected into cells. Transfection efficiencies in PASMCs were verified (Figure 2B). Results indicated that overexpression of miR-593-5p markedly reduced the viability and migration of PASMCs (Figure 2C and 2D). However, opposite results were found in PASMCs transfected with miR-593-5p inhibitor.

PLK1 Was the Target Gene of miR-593-5p

Both the mRNA and protein levels of PLK1 were found to be negatively regulated by miR-593-5p (Figure 3A and 3B). To further explore the interaction between miR-593-5p and PLK1, wild-type, and mutant-type PLK1 sequences were constructed (Figure 3C). Dual-luciferase reporter gene assay demonstrated that luciferase activity of PASMCs was significantly decreased after co-transfection of miR-593-5p mimics and wild-type PLK1 when compared with those co-transfected with miR-593-5p mimics and mutant-type PLK1 (Figure 3D). The above results demonstrated that miR-593-5p could directly bind to PLK1 and negatively regulate its expression.

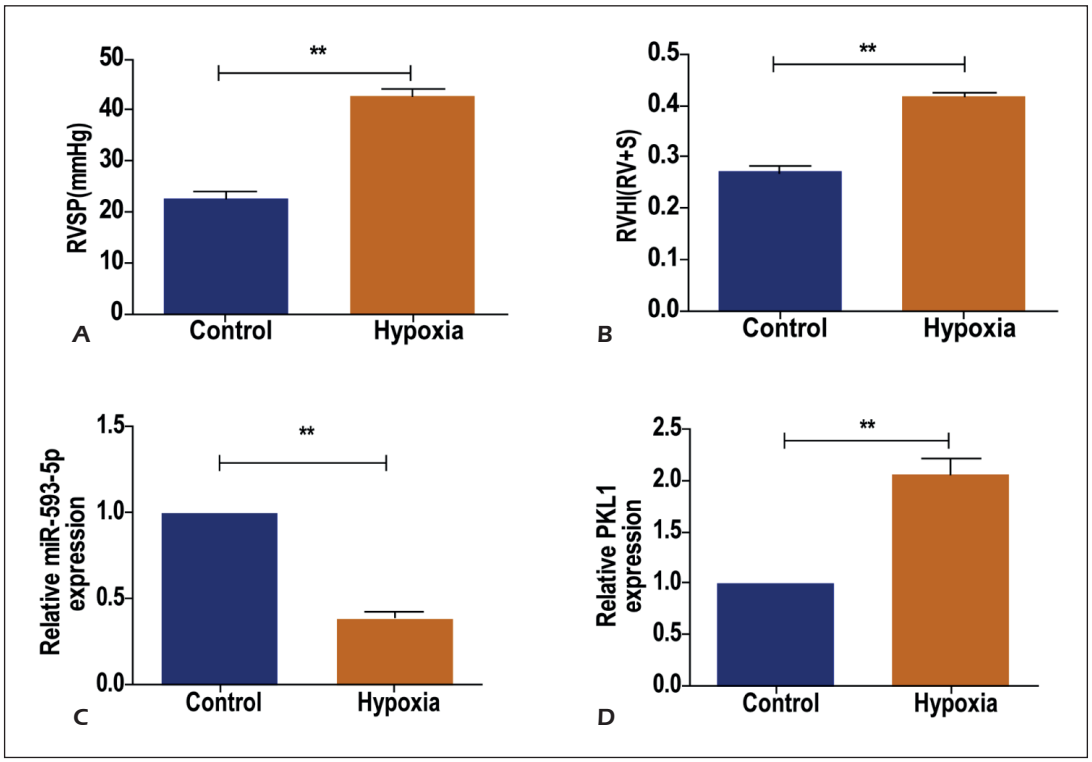

Figure 1. MiR-593-5p was lowly expressed in lung tissues of HPH rats. **A-B**, RVSP (**A**) and RVHI (**B**) in rats of the hypoxic group were significantly elevated when compared with those of the control group. **C-D**, QRT-PCR data showed downregulated miR-593-5p (**C**) and upregulated PLK1 (**D**) in lung tissues of HPH rats than those of controls.

MiR-593-5p Inhibited the Proliferation and Migration of PASMCs Via Targeting PLK1

Rescue experiments were conducted to further verify the role of PLK1 in regulating PASMCs proliferation and migration. Overexpression plasmid of PLK1 was constructed. CCK-8 results showed that inhibited viability of PASMCs induced by miR-593-5p overexpression could be reversed by PLK1 overexpression (Figure 4A). Similarly, decreased migration of PASMCs induced by miR-593-5p overexpression was markedly elevated after PLK1 up-regulation (Figure 4B).

Discussion

PAH is an abnormal hemodynamic disease, which is closely related to pulmonary vasospasm, pulmonary vascular remodeling, and increased pulmonary vascular resistance due to pulmonary thrombosis[15]. PAH occurs in people of different ages, genders, and ethnicities. Some studies have shown that hypoxic pulmonary artery contraction and hypoxic pulmonary vascular remodeling are the main pathological changes of PAH[16]. Therefore, in-depth researches on the potential mechanism of PAH contribute to develop novel therapeutic drugs, eventually improving the clinical outcomes of affected patients effectively.

MiRNAs are highly conserved, single-stranded small-molecule RNAs that are ubiquitous in eukaryotic cells. MiRNAs can regulate various biological functions, such as the development, differentiation, proliferation, and apoptosis of cells[17,18]. It is reported[19,20] that miR-451 and miR-1 are differentially expressed in PAH, which can be utilized as diagnostic markers. Relative studies[21-23] have shown that some certain miRNAs regulate the development of pulmonary diseases *via* activating the abnormal proliferation of PASMCs. In the present study, we found that miR-593-5p was lowly expressed in lung tissues of HPH rats. Moreover, miR-593-5p overexpression significantly inhibited the proliferation and migration of PASMCs, indicating its important role in HPH.

Polo-like kinase 1 (PLK1) is an important serine/threonine kinase[24]. Studies have elucidateded that PLK1 is related to mitotic spindle function[25].

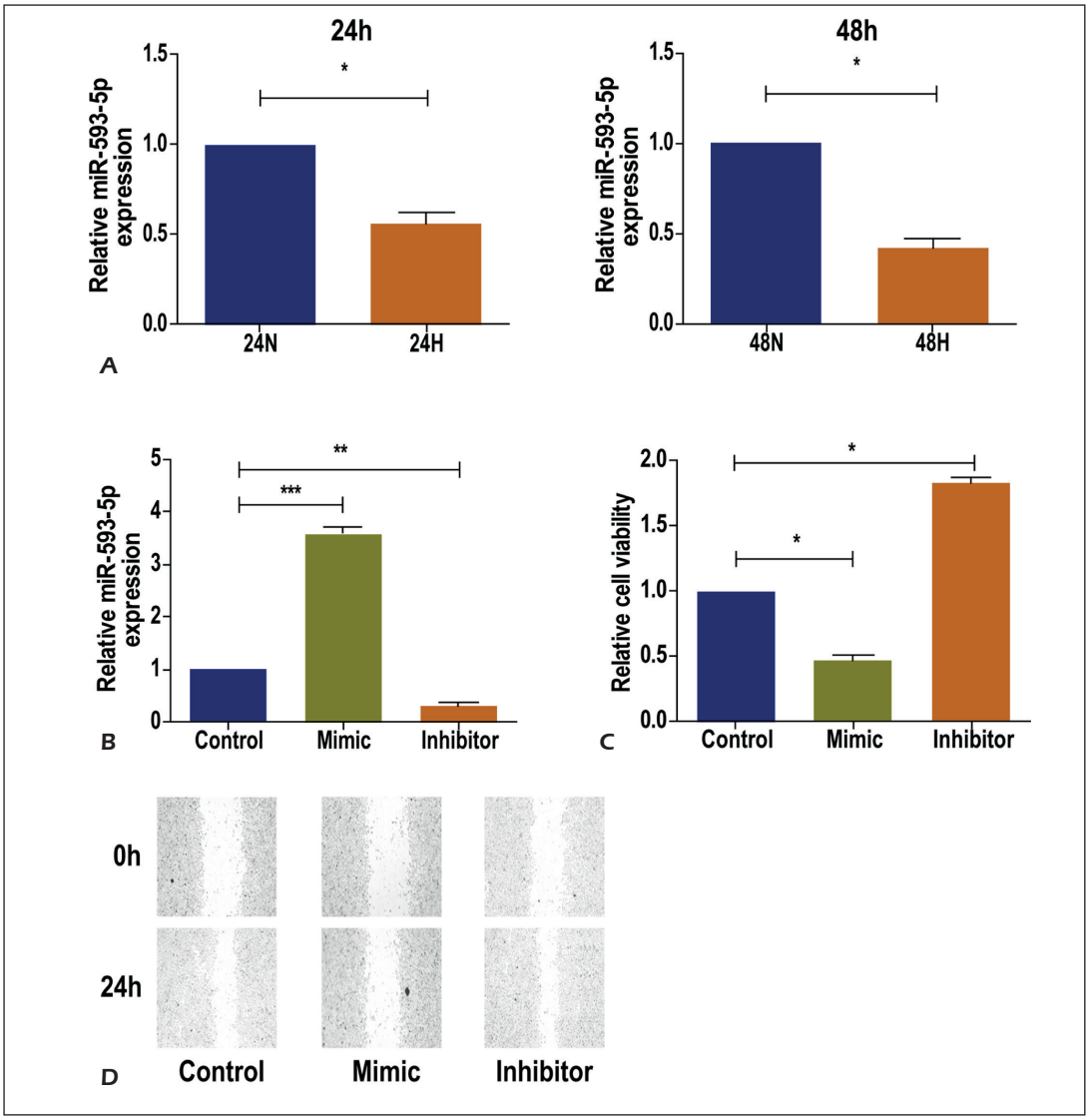

Figure 2. MiR-593-5p inhibited the proliferation and migration of PASMCs. **A**, QRT-PCR results showed that miR-593-5p level in the 24-h hypoxic group and the 48-h hypoxic group was remarkably lower than the control group. **B**, Transfection efficiencies of miR-593-5p mimics and inhibitor in PASMCs. **C-D**, Overexpression of miR-593-5p markedly reduced the viability (**C**) and migration (**D**) of PASMCs.

The mitotic process is associated with the phosphorylation level of heat shock transcription factor 1 (HSF1), which is regulated by PLK1. Previous studies have also pointed out that downregulated PLK1 in oral squamous cell carcinoma cells strongly inhibits cell proliferation[26]. Also, PLK1 knockdown remarkably reduces the migration and invasion of tumor cells[27]. Therefore, PLK1 is considered as a potential target for the treatment of malignant tumors. Our study revealed that the mRNA expression level of PLK1 in lung tissues of HPH rats was remarkably higher than that of controls. PLK1 expression was found to be regulated by miR-593-5p. Moreover, the inhibitory effects of miR-593-5p on the proliferation and migration of PASMCs could be reversed by PLK1 overexpression.

Conclusions

We showed that miR-593-5p is lowly expressed in lung tissues of HPH rats. Also, miR-593-5p stimulates the proliferation and migration of PASMCs *via* targeting PLK1, thereby promoting HPH development.

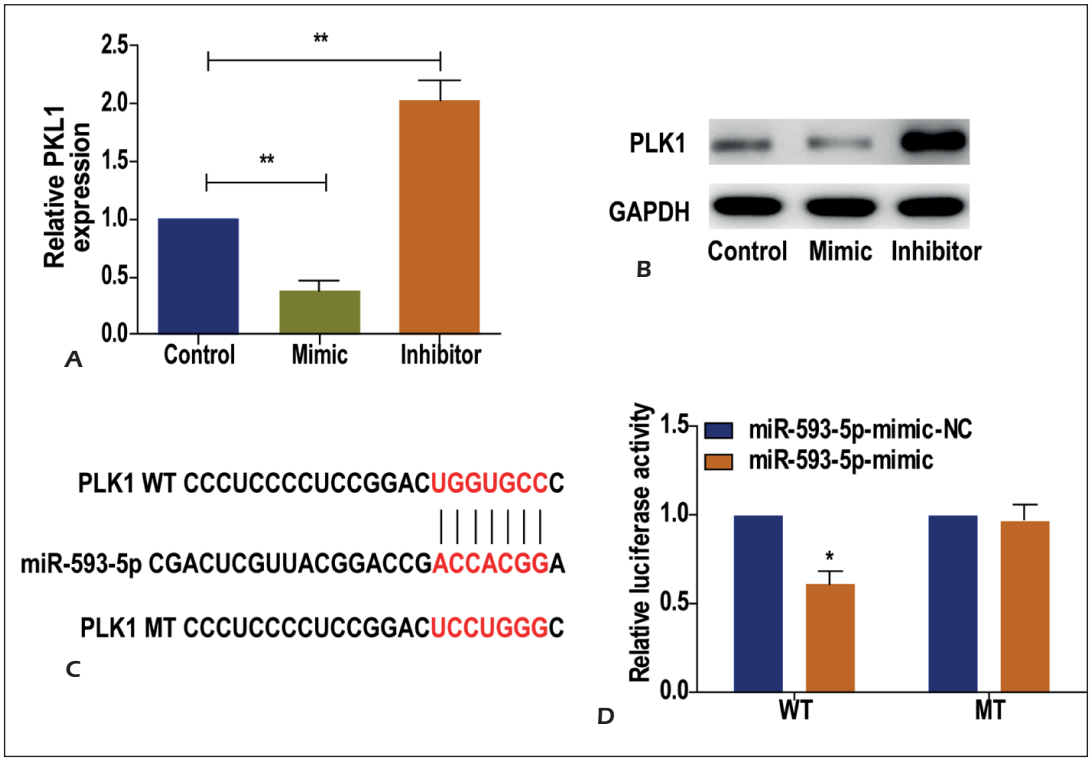

Figure 3. PLK1 was the target gene of miR-593-5p. **A**-**B**, The mRNA (**A**) and protein (**B**) levels of PLK1 were found to be negatively regulated by miR-593-5p. **C**, Construction of wild-type and mutant-type PLK1 sequences. **D**, Luciferase activity of PASMCs was significantly decreased after co-transfection of miR-593-5p mimics and wild-type PLK1 when compared with those co-transfected with miR-593-5p mimics and mutant-type PLK1.

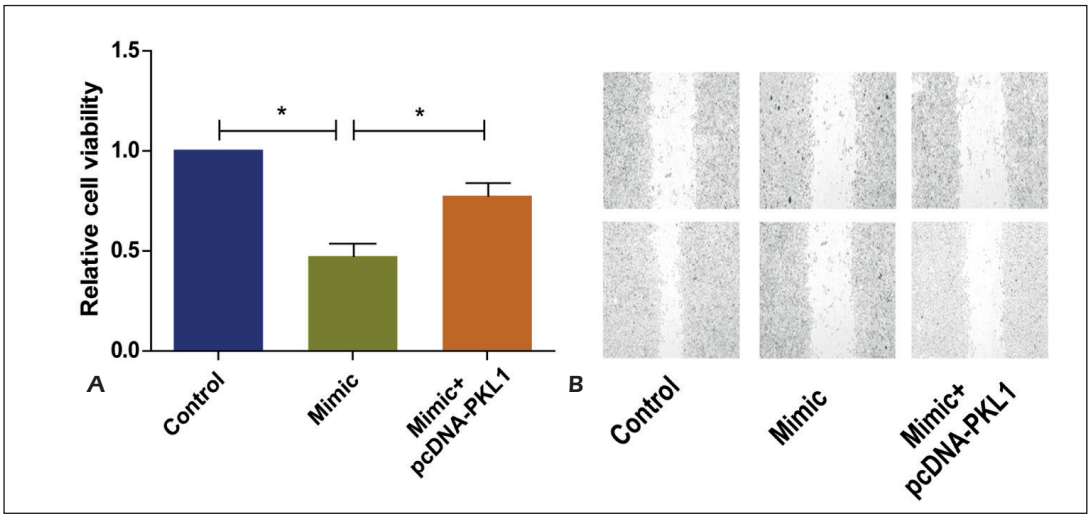

Figure 4. MiR-593-5p inhibited the proliferation and migration of PASMCs via targeting PLK1. **A**, CCK-8 results showed that inhibited viability of PASMCs induced by miR-593-5p overexpression could be reversed by PLK1 overexpression. **C**, Decreased migration of PASMCs induced by miR-593-5p overexpression was markedly elevated after PLK1 overexpression.

Conflict of Interests

The authors declare that they have no conflict of interest.

References

1) Boehme J, Sun X, Tormos KV, Gong W, Kellner M, Datar SA, Kameny RJ, Yuan JX, Raff GW, Fineman JR, Black SM, Maltepe E. Pulmonary artery smooth muscle cell hyperproliferation and metabolic shift triggered by pulmonary overcirculation. Am J Physiol Heart Circ Physiol 2016; 311: H944-H957.
2) Wang AP, Li XH, Yang YM, Li WQ, Zhang W, Hu CP, Zhang Z, Li YJ. A critical role of the mTOR/eIF2alpha pathway in hypoxia-induced pulmonary hypertension. PLoS One 2015; 10: e130806.
3) Kumar S, Wang G, Liu W, Ding W, Dong M, Zheng N, Ye H, Liu J. Hypoxia-induced mitogenic factor promotes cardiac hypertrophy via calcium-dependent and hypoxia-inducible factor-1alpha mechanisms. Hypertension 2018; 72: 331-342.
4) Dabral S, Tian X, Kojonazarov B, Savai R, Ghofrani HA, Weissmann N, Florio M, Sun J, Jonigk D, Maegel L, Grimminger F, Seeger W, Savai PS, Schermuly RT. Notch1 signalling regulates endothelial proliferation and apoptosis in pulmonary arterial hypertension. Eur Respir J 2016; 48: 1137-1149.
5) Kumar R, Mickael C, Kassa B, Gebreab L, Robinson JC, Koyanagi DE, Sanders L, Barthel L, Meadows C, Fox D, Irwin D, Li M, McKeon BA, Riddle S, Dale BR, Morgan LE, Evans CM, Hernandez-Saavedra D, Bandeira A, Maloney JP, Bull TM, Janssen WJ, Stenmark KR, Tuder RM, Graham BB. TGF-beta activation by bone marrow-derived thrombospondin-1 causes Schistosoma- and hypoxia-induced pulmonary hypertension. Nat Commun 2017; 8: 15494.
6) Horita H, Furgeson SB, Ostriker A, Olszewski KA, Sullivan T, Villegas LR, Levine M, Parr JE, Cool CD, Nemenoff RA, Weiser-Evans MC. Selective inactivation of PTEN in smooth muscle cells synergizes with hypoxia to induce severe pulmonary hypertension. J Am Heart Assoc 2013; 2: e188.
7) Hurst LA, Dunmore BJ, Long L, Crosby A, Al-Lamki R, Deighton J, Southwood M, Yang X, Nikolic MZ, Herrera B, Inman GJ, Bradley JR, Rana AA, Upton PD, Morrell NW. TNFalpha drives pulmonary arterial hypertension by suppressing the BMP type-II receptor and altering NOTCH signalling. Nat Commun 2017; 8: 14079.
8) Gao W, Shao R, Zhang X, Liu D, Liu Y, Fa X. Up-regulation of caveolin-1 by DJ-1 attenuates rat pulmonary arterial hypertension by inhibiting TGFbeta/Smad signaling pathway. Exp Cell Res 2017; 361: 192-198.
9) Maute RL, Schneider C, Sumazin P, Holmes A, Califano A, Basso K, Dalla-Favera R. tRNA-derived microRNA modulates proliferation and the DNA damage response and is down-regulated in B cell lymphoma. Proc Natl Acad Sci U S A 2013; 110: 1404-1409.
10) Gong J, Zhang JP, Li B, Zeng C, You K, Chen MX, Yuan Y, Zhuang SM. MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R. Oncogene 2013; 32: 3071-3079.
11) Wallace E, Morrell NW, Yang XD, Long L, Stevens H, Nilsen M, Loughlin L, Mair KM, Baker AH, MacLean MR. A sex-specific microRNA-96/5-hydroxytryptamine 1B Axis influences development of pulmonary hypertension. Am J Respir Crit Care Med 2015; 191: 1432-1442.
12) Dang LT, Lawson ND, Fish JE. MicroRNA control of vascular endothelial growth factor signaling output during vascular development. Arterioscler Thromb Vasc Biol 2013; 33: 193-200.
13) Deng B, Du J, Hu R, Wang AP, Wu WH, Hu CP, Li YJ, Li XH. MicroRNA-103/107 is involved in hypoxia-induced proliferation of pulmonary arterial smooth muscle cells by targeting HIF-1beta. Life Sci 2016; 147: 117-124.
14) Zhang WF, Xiong YW, Zhu TT, Xiong AZ, Bao HH, Cheng XS. MicroRNA let-7g inhibited hypoxia-induced proliferation of PASMCs via G0/G1 cell cycle arrest by targeting c-myc. Life Sci 2017; 170: 9-15.
15) Lewis GD, Bossone E, Naeije R, Grunig E, Saggar R, Lancellotti P, Ghio S, Varga J, Rajagopalan S, Oudiz R, Rubenfire M. Pulmonary vascular hemodynamic response to exercise in cardiopulmonary diseases. Circulation 2013; 128: 1470-1479.
16) Mao SZ, Fan XF, Xue F, Chen R, Chen XY, Yuan GS, Hu LG, Liu SF, Gong YS. Intermedin modulates hypoxic pulmonary vascular remodeling by inhibiting pulmonary artery smooth muscle cell proliferation. Pulm Pharmacol Ther 2014; 27: 1-9.
17) Sheng ZX, Yao H, Cai ZY. The role of miR-146b-5p in TLR4 pathway of glomerular mesangial cells with lupus nephritis. Eur Rev Med Pharmacol Sci 2018; 22: 1737-1743.
18) Kim J. MicroRNAs as critical regulators of the endothelial to mesenchymal transition in vascular biology. BMB Rep 2018; 51: 65-72.
19) Song XW, Zou LL, Cui L, Li SH, Qin YW, Zhao XX, Jing Q. Plasma miR-451 with echocardiography serves as a diagnostic reference for pulmonary hypertension. Acta Pharmacol Sin 2018; 39: 1208-1216.
20) Sysol JR, Chen J, Singla S, Zhao S, Comhair S, Natarajan V, Machado RF. Micro-RNA-1 is decreased by hypoxia and contributes to the development of pulmonary vascular remodeling via regulation of sphingosine kinase 1. Am J Physiol Lung Cell Mol Physiol 2018; 314: L461-L472.
21) Feng Q, Tian T, Liu J, Zhang L, Qi J, Lin X. Deregulation of microRNA31a5p is involved in the development of primary hypertension by suppressing apoptosis of pulmonary artery smooth muscle cells via targeting TP53. Int J Mol Med 2018; 42: 290-298.
22) Zhang X, Shao R, Gao W, Sun G, Liu Y, Fa X. Inhibition of miR-361-5p suppressed pulmonary artery smooth muscle cell survival and migration by

targeting ABCA1 and inhibiting the JAK2/STAT3 pathway. Exp Cell Res 2018; 363: 255-261.

23) Feng Q, Tian T, Liu J, Zhang L, Qi J, Lin X. Deregulation of microRNA31a5p is involved in the development of primary hypertension by suppressing apoptosis of pulmonary artery smooth muscle cells via targeting TP53. Int J Mol Med 2018; 42: 290-298.

24) Schmucker S, Sumara I. Molecular dynamics of PLK1 during mitosis. Mol Cell Oncol 2014; 1: e954507.

25) Pidoux AL, LeDizet M, Cande WZ. Fission yeast pkl1 is a kinesin-related protein involved in mitotic spindle function. Mol Biol Cell 1996; 7: 1639-1655.

26) Kim SA, Kwon SM, Yoon JH, Ahn SG. The antitumor effect of PLK1 and HSF1 double knockdown on human oral carcinoma cells. Int J Oncol 2010; 36: 867-872.

27) Nakamura Y, Fujimoto M, Fukushima S, Nakamura A, Hayashida N, Takii R, Takaki E, Nakai A, Muto M. Heat shock factor 1 is required for migration and invasion of human melanoma in vitro and in vivo. Cancer Lett 2014; 354: 329-335.