Int J Clin Exp Med 2020;13(6):4507-4516
www.ijcem.com /ISSN:1940-5901/IJCEM0109306

Review Article

Recombinant human erythropoietin promotes the proliferation and migration of vascular smooth muscle cells via miR-26a-5p targeting the PTEN signaling pathway

Hongping Shan[1], Li Wang[2], Huimin Jin[1]

[1]*Division of Nephrology, Shanghai Pudong Hospital, Fudan University, Pudong Medical Center, Shanghai, China;*
[2]*Department of General Surgery, Shanghai Public Health Clinical Center, Shanghai, China*

Received February 16, 2020; Accepted March 23, 2020; Epub June 15, 2020; Published June 30, 2020

Abstract: This paper aims to explore how recombinant human erythropoietin (rHuEPO) regulates the proliferation and migration of vascular smooth muscle cells (VSMCs). After rHuEPO was used to treat VSMCs, the miR-26a-5p expression in the cells was quantified using qPCR. The cell proliferation, apoptosis, the cell cycle, and the invasion and migration were quantified using CCK-8, flow cytometry, and Transwell, respectively. The PTEN signaling pathway proteins were determined using Western blot (WB). The regulatory relationship between miR-26a-5p and PTEN in the cells was analyzed. rHuEPO can inhibit the proliferation, invasion, and migration of VSMCs, and it can increase their apoptotic rate. It can increase miR-26a-5p expression in the cells thereby reducing the PTEN expression. It can increase the miR expression, thereby inhibiting the cells' proliferation, invasion, and migration, and increasing their apoptotic rate. It can inhibit and reduce the PTEN expression, thereby inhibiting the cells' proliferation, invasion, and migration, and increasing their apoptotic rate. A dual luciferase reporter gene assay (DLRGA) confirmed the targeted relationship of miR-26a-5p with PTEN. VSMCs in the miR-26a-5p-mimics + PTEN-inhibitor group were not different from those in the si-NC group in terms of their proliferation, invasion, migration, and apoptosis, but their malignant phenotypes remarkably increased, and their apoptotic rate was remarkably reduced compared with the miR-26a-5p-mimics group. In conclusion, rHuEPO promotes the proliferation and migration of VSMCs through miR-26a-5p and the PTEN signaling pathway.

Keywords: rHuEPO, miR-26a-5p, PTEN signaling pathway, VSMCs, cell proliferation, cell migration

Introduction

Vascular smooth muscle cells (VSMCs) exert a crucial function in the pathogenesis of various cardiovascular diseases [1], because their proliferation and migration are the most critical reasons for disease progression [2]. Moreover, their growth and migration lead to various harmful stimuli, resulting in pathological and physiological arterial remodeling as well as plaque formation, thereby causing vascular thickening and narrowing and blood flow obstruction. Eventually, cardiovascular diseases occur [3]. As a glycoprotein hormone that stimulates erythroid cells to proliferate, differentiate, and survive [4], recombinant human erythropoietin (rHuEPO) has a great effect on tissue development and protection, and its known anti-apoptotic effect improves the neurodevelopmental outcomes of premature infants [5, 6]. According to Park et al., this hormone can lead to hypertrophy and hyperplasia, and it can induce VSMCs to proliferate. Erythropoietin (EPO) expression can induce the cells to migrate and invade by activating NF-kB to bind to AP-1 and through MMP-9 expression [7]. In this study, the proliferation and migration mechanisms of VSMCs were integrated to explore the potential regulatory mechanism of rHuEPO, so as to find new therapeutic targets for VSMCs.

As non-coding RNA molecules, miRNAs that regulate gene expression through a variety of mechanisms can control smooth muscle cell

phenotypes and arterial contractility [8, 9]. Studies have reported that they play a crucial and regulatory role in VSMCs. For example, miR-362-3p inhibits the cells from proliferating and migrating by targeting ADAMTS1 [10], and miR-26a-5p may be involved in the calls' disease progression. According to Tan et al., miR-26a is an important regulator for the neointimal hyperplasia of VSMC functions [11], and its up-regulation before β-GP therapy can inhibit VSMC calcification by targeting CTGF, according to Wu et al. [12]. PTEN is a phosphatase whose overexpression inhibits cell migration, which can be enhanced by antisense PTEN [13]. This phosphatase plays a big role in inducing cell cycle arrest, programmed cell apoptosis, and cell physiological behaviors (such as migration and adhesion) [14]. Moreover, its abnormally high expression in VSMCs suggests that it may be involved in the pathological process of the cells [15].

The regulatory mechanisms of rHuEPO, miR-26a-5p, and PTEN in VSMCs have been rarely studied, and whether rHuEPO plays a protective role by mediating miR-26a-5p and PTEN remains to be further explored.

Methods

Cell culture and transfection

Purchased from ATCC, United States, the VSMCs (BNCC332960) were placed in an RPMI 1640 medium [10% fetal bovine serum (FBS)] and cultured in an incubator (37°C, 5% CO_2).

Main instruments and reagents

The PMI 1640 medium (UFC11875) was purchased from Shanghai Junrui Biotechnology Co., Ltd. The DMEM medium (BH-S3208) was purchased from Shanghai Bohu Biotechnology Co., Ltd. The TRIzol kits (hz81027-501) were purchased from Shanghai Huzhen Industrial Co., Ltd. The reverse transcription kits were purchased from GeneCopoeia, USA. The PrimeScript RT Master Mix (YT378-XQM) was purchased from the Beijing Biolab Science and Technology Co., Ltd. The RIPA buffer (R1176) was purchased from the Shanghai Shifeng Biological Technology Co., Ltd. The BCA protein assay kits (JK-201) were purchased from the Shanghai Jingke Chemical Technology Co., Ltd. The PTEN (H00005728-P01) was purchased from the Shanghai Kemin Biological Technology Co., Ltd. The secondary antibody (C-8250) was purchased from the Shanghai Caiyou Industry Co., Ltd. The Lipofectamine™ 2000 (11668019) was purchased from the Shanghai Mito Biological Technology Co., Ltd. The dual luciferase reporter gene assay (DLRGA) kits (CDLG-4997) were purchased from Wuhan Purity Biotechnology Co., Ltd. The Pierce™ Magnetic RNA-Protein Pull-Down Kits (20164) were purchased from Shanghai Beinuo Biotechnology Co., Ltd. Annexin V-FITC (DXT-130-097-928) was purchased from the Shanghai Kemin Biological Technology Co., Ltd. The CCK-8 kits (IC-CCK8-Hu) were purchased from Shanghai Yubo Biotech Co., Ltd. Transwell kit (Transwell) was purchased from BioGenius, Shanghai, China. The flow cytometer was purchased from BD, USA. The multifunctional microplate reader (DLK0001622) was purchased from BioTek, USA. All the primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd.

qRT-PCR

The TRIzol kit was used to draw RNA from the VSMCs, whose purity, concentration, and integrity were determined using an UV spectrophotometer and agarose gel electrophoresis. The drawn RNA was reversely transcribed into cDNA based on the instructions of the reverse transcription kit (Thermo Fisher Scientific). A SYBR Premix Ex Taq TM kit (Takara Biotechnology Co., Ltd.) was used for the reaction on a PCR instrument (ABI, 7500 PCR instrument), with GAPDH or U6 as an internal reference (**Table 1**). The Conditions for the PCR amplification and cycle were pre-denaturation (95°C, 10 min), denaturation (95°C, 15 s), and annealing/extension (60°C, 60 s), cycling 40 times. The data were obtained after 3 repeated experiments, and the relative expression was calculated using $2^{-\Delta\Delta CT}$.

Western blotting (WB)

The cultured cells were lysed with a RIPA buffer, and the protein concentration was measured with a BCA protein assay kit. The protein was separated with 12% SDS-PAGE to transfer it to the PVDF membrane (0.22 μm) after its concentration was adjusted to 4 μg/μL. The membrane was sealed in 5% skimmed milk (2 hours), and then mixed with PTEN

Table 1. Primer sequences

	Forward	Reverse
miR-26a-5p	5'-GGATCCGCAGAAACTCCAGAGA-3'	5'-TTGGAGGAAAGACGATTTCCGT-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
PTEN	5'-ACCAGTGGCACTGTTGTTTCAC-3'	5'-TTCCTCTGGTCCTGGTATGAAG-3'
GAPDH	5'-CCGTATTCAGCATTCTATGCTCTC-3'	5'-TGGATACACACTCTGGGGCT-3'

(1:1000) for sealing (4°C) overnight. After cleaning it to remove the primary antibody, it was mixed with the secondary antibody [goat anti-rabbit (1:5000)] labeled by horseradish peroxidase, incubated at 37°C (1 hour), and then rinsed three times with PBS (5 min each time). Finally, it was luminesced with the ECL reagent and it was developed after the excess liquid on it was blotted dry with filter paper. The protein bands were scanned, and their gray values were analyzed in Quantity One, with GAPDH as the internal reference.

CCK-8 to determine the cell proliferation

A CCK-8 assay kit was used for the quantification. The cells were collected after a 24-hour transfection, then adjusted to 4×10^6 cells/well, and finally inoculated on a 96-well plate. After 24-, 48-, 72-, and 96-hours of culturing, the CCK-8 solution (10 μL) and the basic medium (DMEM, 90 μL) were added to each well for 2-hours of culturing at 37°C. Next, the optical value (OD) value in each group was measured at 450 nm using a microplate reader.

The cell migration and invasion experiments

The cells were first digested with trypsin, and then resuspended in a serum-free medium. 200 μL of the resuspension was taken out for the cell migration assay, with approximately 5×10^4 cells placed in the upper chamber of the Transwell. Next, another 200 μL of the resuspension was taken out for the cell invasion assay, with the lower chamber of the 6-well plate (containing approximately 5×10^4 cells) added to the FBS-containing medium (1 mL). After the 24-hour conventional culture, the cells in the upper chamber of the Transwell were wiped off with cotton swabs, and those migrating to the lower one were stained with 4% paraformaldehyde and 0.1% crystal violet. After the Transwell chamber was dried, the membrane was prepared and the film was sealed. The cells penetrating the membrane were observed and counted under an optical microscope. Three repeated experiments were conducted.

The cell apoptosis experiment

According to the apoptosis kit instructions for the Annexin V-FITC/PI double staining, flow cytometry was used to determine the cell apoptotic rate in each group. After being digested with trypsin and cleaned twice with PBS, they were collected into centrifuge tubes. A buffer solution (1 mL) was added with the Annexin-V-FITC labeling solution (20 μL) and the PI reagent (20 μL), and then it was incubated in the dark (at room temperature for 5 min). The flow cytometer was used for the quantification. The experiment was conducted three times to obtain the average value.

Dual luciferase reporter gene assay (DLRGA)

TargetScan was used to predict the binding sites of miR-26a-5p and PTEN. Subsequently, a fragment of the PTEN 3'-untranslated region (3'-UTR) containing the predicted binding site wild type (PTEN Wt) or the mutant (PTEN Mut) was cloned on the vectors. After DNA sequencing for verification, the plasmids (miR-26a-5p-mimics, NC-mimics) were transfected into the VSMCs based on the Lipofectamine™ 2000 instructions (Invitrogen, USA). The cells were collected after the 48-hour transfection. The dual luciferase reporter gene assay system (DLR® (Promega Corporation)) was used for the analysis.

Statistical analysis

In this study, SPSS 20.0 was used to statistically analyze the collected data. GraphPad 7 was used to plot the required figures. Comparison between two groups were conducted using t tests, and comparisons between multiple groups were conducted using one-way analyses of variance, and the post hoc pairwise comparisons were conducted using LSD-t

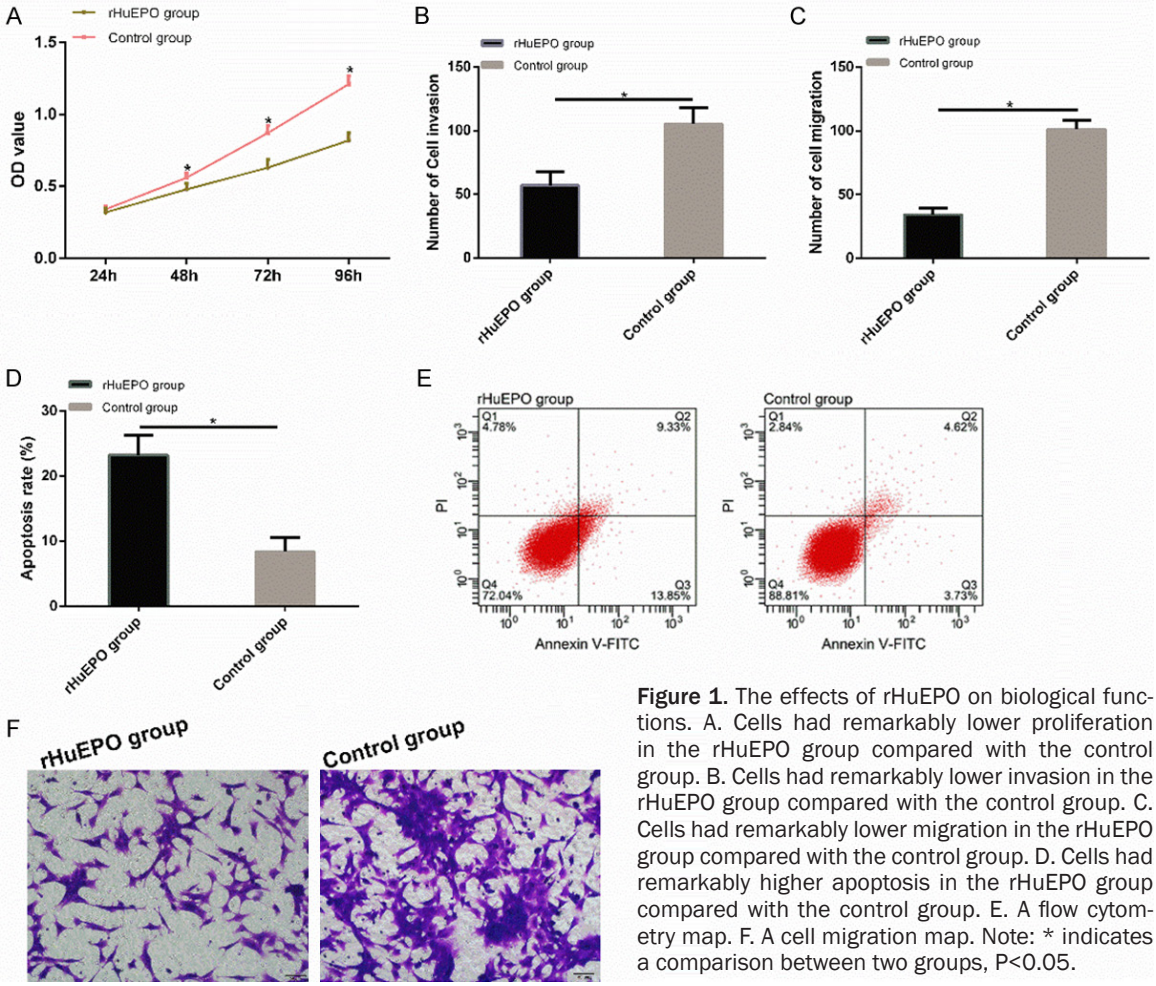

Figure 1. The effects of rHuEPO on biological functions. A. Cells had remarkably lower proliferation in the rHuEPO group compared with the control group. B. Cells had remarkably lower invasion in the rHuEPO group compared with the control group. C. Cells had remarkably lower migration in the rHuEPO group compared with the control group. D. Cells had remarkably higher apoptosis in the rHuEPO group compared with the control group. E. A flow cytometry map. F. A cell migration map. Note: * indicates a comparison between two groups, P<0.05.

tests. When P<0.05, the difference was statistically significant.

Results

The effects of rHuEPO on biological functions

The effects of rHuEPO on the biological functions of the treated VSMCs were studied. The cells had remarkably lower proliferation, invasion, and migration, but a remarkably higher apoptotic rate in the rHuEPO group compared with the control group (P<0.05). See **Figure 1**.

The effects of rHuEPO on miR-26a-5p and the PTEN signaling pathway

To determine whether rHuEPO affected miR-26a-5p and the PTEN signaling pathway, the miR-26a-5p and p-PTEN expressions in the rHuEPO and control groups were determined. Compared with the control group, the miR-26a-5p expression increased remarkably, but the p-PTEN content was remarkably decreased in the rHuEPO group (P<0.05). See **Figure 2**.

The effects of miR-26a-5p on the biological functions

After transfection with the miR-26a-5p-mimics and after transfecting miR-NC into VSMCs, the miR-26a-5p expression in the miR-26a-5p-mimics group was remarkably higher than it was in the miR-NC group (P<0.05). Additionally, the cells had remarkably reduced proliferation, invasion, and migration, but a remarkably increased apoptotic rate in the miR-26a-5p-mimics group compared with the miR-NC group (P< 0.05). See **Figure 3**.

Effects of PTEN on biological functions

After transfection with the PTEN-inhibitor and si-NC into VSMCs, the PTEN mRNA expression

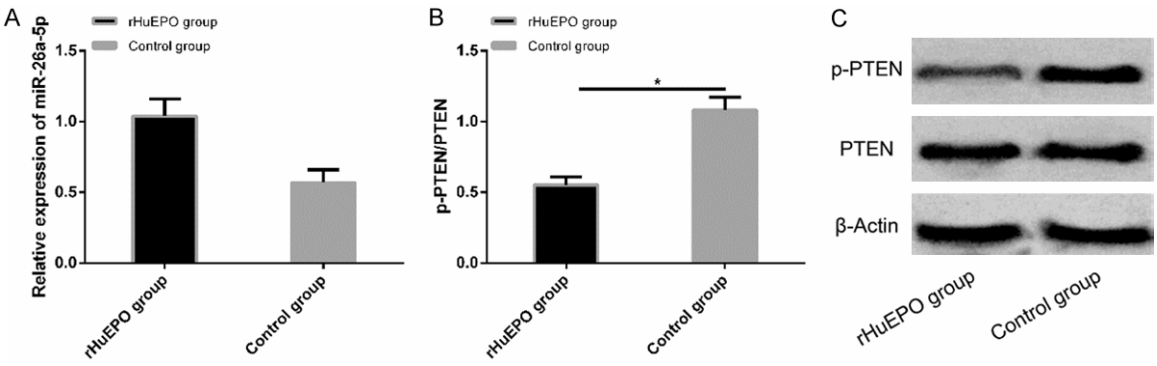

Figure 2. The effects of rHuEPO on the miR-26a-5p and PTEN signaling pathways. A. The miR-26a-5p expression was remarkably higher in the rHuEPO group. B. The miR-26a-5p expression was remarkably lower in the rHuEPO group. C. Corresponding protein figure. Note: * indicates a comparison between two groups, P<0.05.

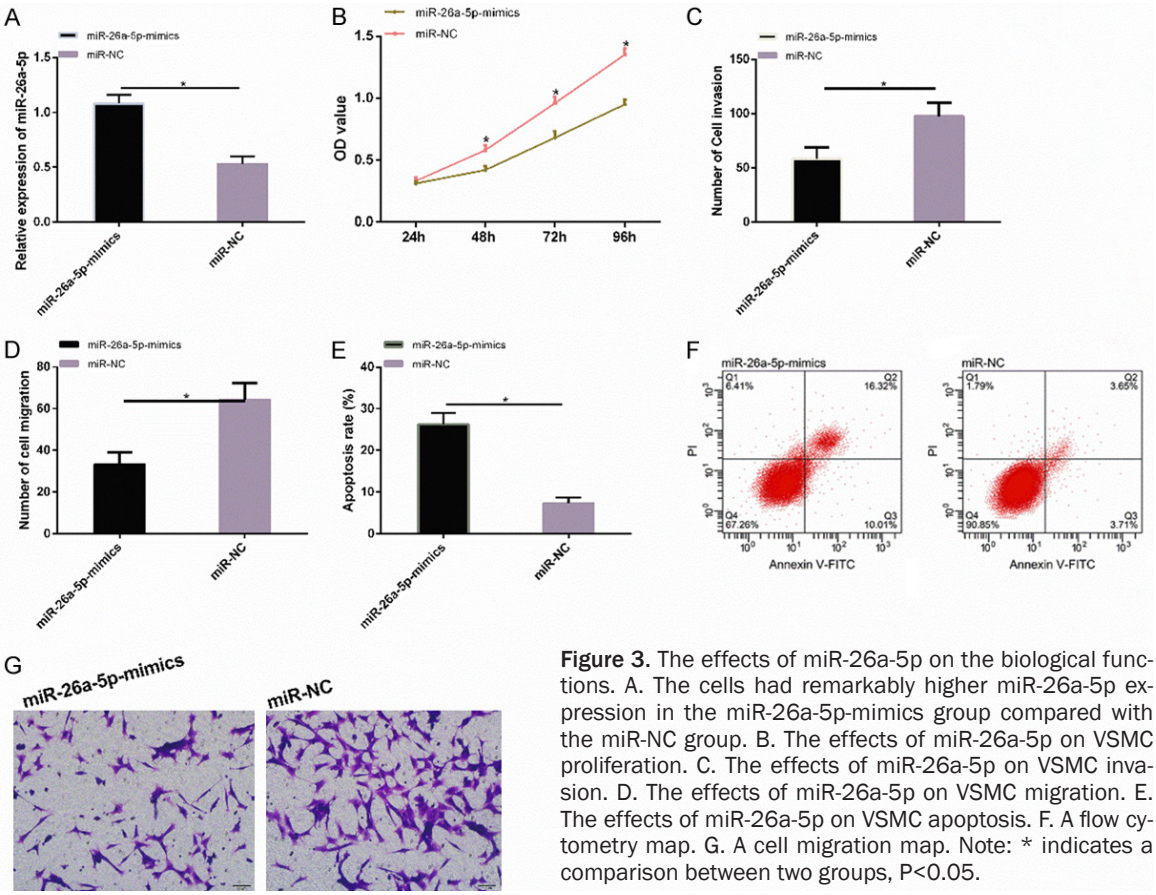

Figure 3. The effects of miR-26a-5p on the biological functions. A. The cells had remarkably higher miR-26a-5p expression in the miR-26a-5p-mimics group compared with the miR-NC group. B. The effects of miR-26a-5p on VSMC proliferation. C. The effects of miR-26a-5p on VSMC invasion. D. The effects of miR-26a-5p on VSMC migration. E. The effects of miR-26a-5p on VSMC apoptosis. F. A flow cytometry map. G. A cell migration map. Note: * indicates a comparison between two groups, P<0.05.

was inhibited and remarkably lower in the PTEN-inhibitor group compared with the si-NC group (P<0.05). Additionally, the cells had remarkably reduced proliferation, invasion, and migration, but a remarkably higher apoptotic rate in the PTEN-inhibitor group compared with the si-NC group (P<0.05). See **Figure 4**.

The targeted relationship between miR-26a-5p and PTEN

According to the above results, miR-26a-5p may mediate the effects of rHuEPO on the PTEN signaling pathway. Therefore, we determined the possible targets using the predic-

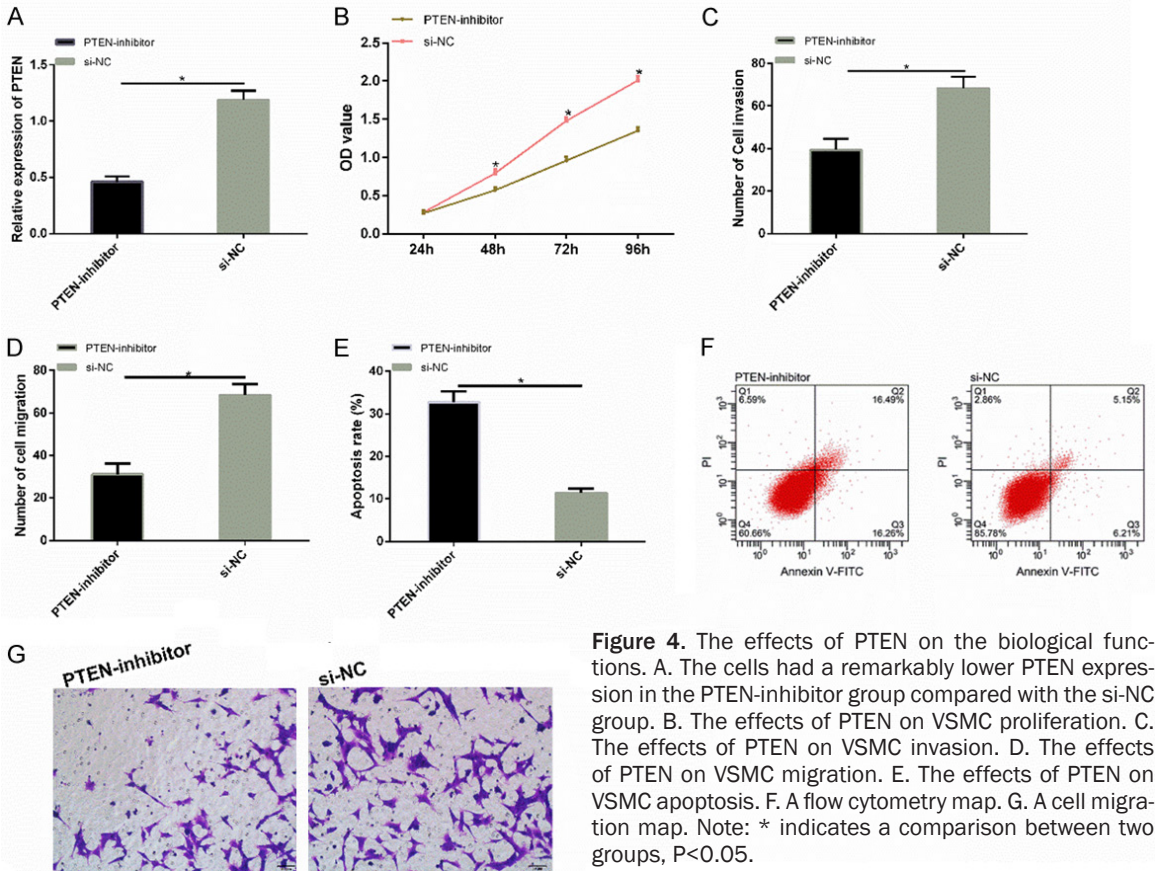

Figure 4. The effects of PTEN on the biological functions. A. The cells had a remarkably lower PTEN expression in the PTEN-inhibitor group compared with the si-NC group. B. The effects of PTEN on VSMC proliferation. C. The effects of PTEN on VSMC invasion. D. The effects of PTEN on VSMC migration. E. The effects of PTEN on VSMC apoptosis. F. A flow cytometry map. G. A cell migration map. Note: * indicates a comparison between two groups, P<0.05.

tion software to explore the possible biological functions of miR-26a-5p. There was a complementary pairing region between miR-26a-5p and the PTEN sequence, which indicates that PTEN is the target gene of miR-26a-5p. After transfection with the miR-26a-5p-mimics, the PTEN-Wt fluorescence activity was remarkably reduced (P<0.05). Compared with the miR-NC group, the expression and content of PTEN increased remarkably in the miR-26a-5p-mimics group (P<0.05). See **Figure 5**.

Rescue experiment

After co-transfection with the miR-26a-5p-mimics + PTEN-inhibitor into VSMCs, their biological functions were determined. The cells in the miR-26a-5p-mimics + PTEN-inhibitor group were not different from those in the si-NC group with respect to their proliferation, invasion, migration, and apoptosis, but their malignant phenotypes increased remarkably, and their apoptotic rates were remarkably reduced compared with the corresponding values in the miR-26a-5p-mimics group. See **Figure 6**.

Discussion

The proliferation and differentiation of VSMCs lead to the development and progression of restenosis and other cardiovascular diseases [16, 17], important for maintaining vascular tone and vascular remodeling [18]. The cells sense the tension stress, applied force, and structure, thereby affecting the pathological and physiological responses of the vascular system [19]. Therefore, we will focus on finding new therapeutic targets for VSMCs.

rHuEPO is a hematopoietic cytokine, and half of its molecular weight consists of sugar moieties that protect it from degradation. rHuEPO regulates red blood cell production by binding to specific membrane receptors and preventing the hypoxia-induced apoptosis of erythroid progenitor cells [20]. More and more scholars have reported on this cytokine in treating VSMCs. For instance, rHuEPO treatment induces changes in pressure factors, increases cytosolic calcium in platelets and VSMCs, and changes the calcium ion influx, which contributes to the con-

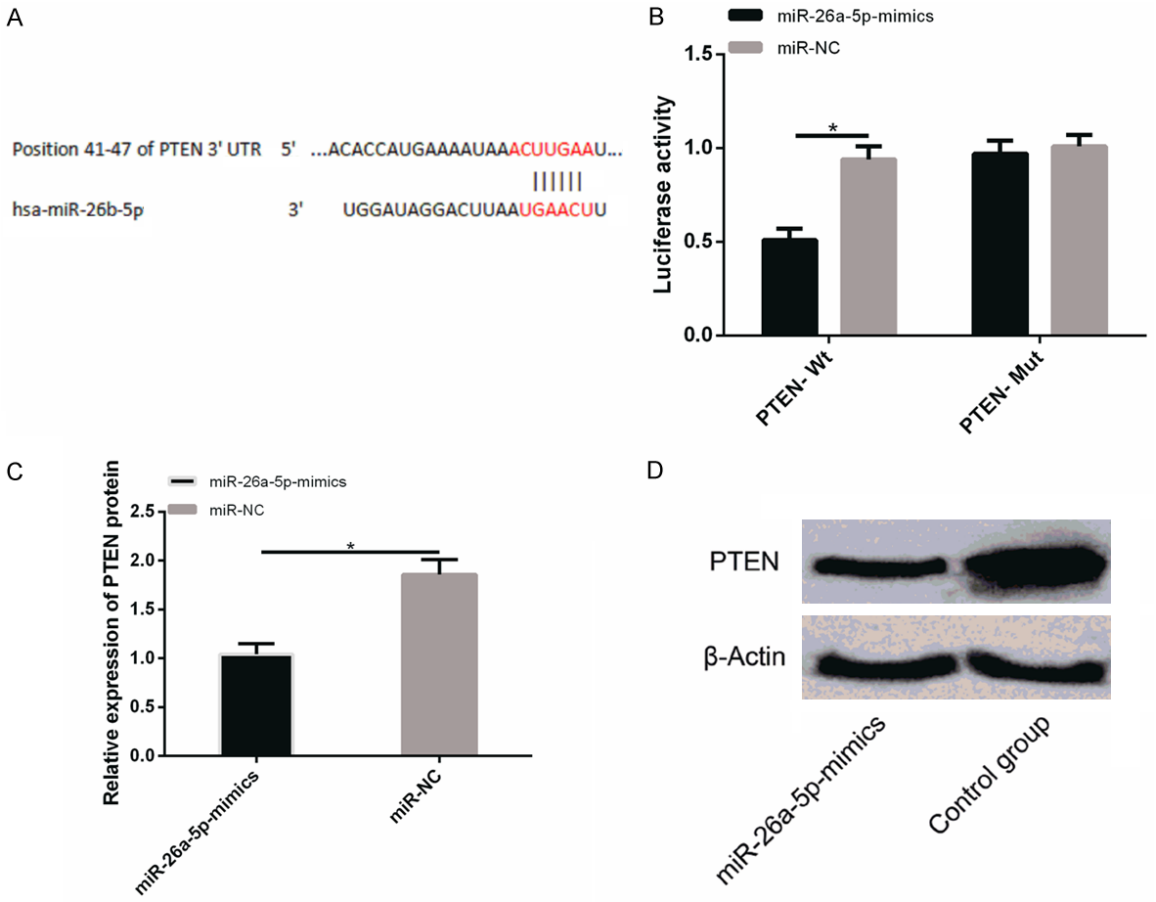

Figure 5. Targeted relationship between miR-26a-5p and PTEN. A. The binding site of miR-26a-5p and PTEN. B. DLRGA results. C. The effects of the miR-26a-5p-mimics on PTEN. D. A protein map of the miR-26a-5p-mimics' effects on PTEN. Note: * indicates a comparison between two groups, P<0.05.

traction of arteriole blood vessels [21]. This treatment functions as a growth promoting factor of VSMCs through the activation of phospholipase C cascade and the expression of protooncogenes [22]. In our study, VSMCs treated by rHuEPO had remarkably lower proliferation, invasion, and migration, but a remarkably higher apoptotic rate compared with the corresponding values in the control group. This indicates that rHuEPO treatment can inhibit VSMCs from proliferating, invading, and migrating, but it increases their apoptotic rate.

miRNAs regulate a variety of biological and pathological processes such as cell proliferation and differentiation, and their imbalance usually leads to cell function decline and disease progression [23]. Therefore, miRNAs are crucial to VSMC development and proliferation. For example, miR-32 regulates the calcification of VSMCs by targeting the PTEN/Akt/RUNX2 axis [24]. Therefore, we first analyzed the miR-26a-5p and PTEN expressions in VSMCs after rHuEPO treatment. rHuEPO can increase miR-26a-5p expression, but it reduces the expression of PTEN phosphorylation in cells. Previous studies have confirmed that various drugs for VSMC therapy exert a protective function through the PTEN signaling pathway. According to Chen et al., the miR-132 targeting of this pathway may be an important regulator for mediating VSMC differentiation by cilostazol [25]. We speculated that rHuEPO might mediate this pathway to improve VSMC proliferation and migration, so we quantified its relevant indicators for verification. In our study, rHuEPO was found to increase miR-26a-5p expression. rHuEPO-treated VSMCs were transfected with miR-26a-5p-mimics and a PTEN-inhibitor, and the cell proliferation, invasion, and migration were inhibited after the transfection with miR-26a-5p. This suggests that up-regulating the

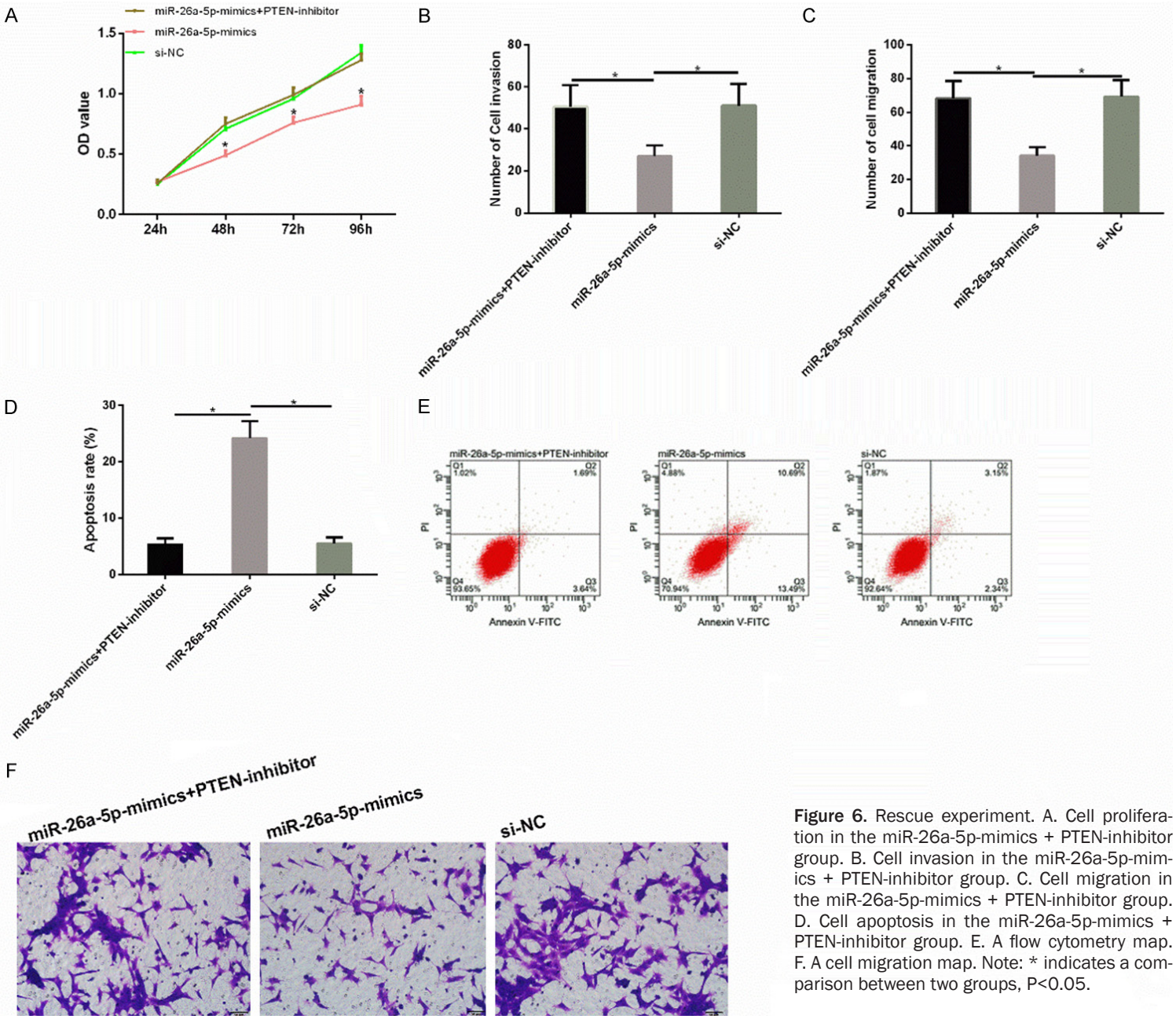

Figure 6. Rescue experiment. A. Cell proliferation in the miR-26a-5p-mimics + PTEN-inhibitor group. B. Cell invasion in the miR-26a-5p-mimics + PTEN-inhibitor group. C. Cell migration in the miR-26a-5p-mimics + PTEN-inhibitor group. D. Cell apoptosis in the miR-26a-5p-mimics + PTEN-inhibitor group. E. A flow cytometry map. F. A cell migration map. Note: * indicates a comparison between two groups, $P<0.05$.

miR-26a-5p expression can inhibit the malignant phenotypes of VSMCs and improve their apoptosis. rHuEPO can inhibit PTEN expression and down-regulate its phosphorylation level. VSMCs had remarkably lower proliferation, invasion, and migration, but improved apoptosis after transfection with TPEN. This indicates that miR-26a-5p and PTEN may be potential targets for VSMC therapy. Based on the above research results, rHuEPO has a great effect on the PTEN signaling pathway during VSMC therapy. It inhibits VSMCs from proliferating, invading, and migrating, and it improves their apoptosis by regulating this pathway. Finally, we confirmed through DLRGA that PTEN is the target gene of miR-26a-5p, and the PTEN-Wt fluorescence activity was remarkably reduced after miR-26a-5p overexpression. The PTEN expression was remarkably reduced after its transfection with the miR-26a-5p-mimics, indicating that there is a targeted regulatory relationship between this miR and PTEN. In addition, we further verified the relationship through co-transfection experiments. The VSMCs in the miR-26a-5p-mimics + PTEN-inhibitor group were not different from those in the si-NC group in terms of their proliferation, invasion, migration, and apoptosis, but their malignant phenotypes increased remarkably, and their apoptotic rate was remarkably reduced compared with the corresponding values in the miR-26a-5p-mimics group. This once again confirms the targeted regulatory relationship.

This study found the regulatory mechanism of rHuEPO in VSMCs, and revealed that miR-26a-5p and PTEN may become potential therapeutic targets for the disease. However, there is still room for improvement. First of all, we can carry out tumor formation in nude mice and supplement whether there is a dose-dependent relationship between rHuEPO and the regulatory mechanism. Moreover, we can analyze additional potential mechanisms of rHuEPO using bioinformatics, and collect more types (cell lines) and different types of (serum) samples, so as to verify our research results.

Acknowledgements

This study was financially supported by the Shanghai Science and Technology Commission (15ZR1437400).

Disclosure of conflict of interest

None.

Address correspondence to: Huimin Jin, Division of Nephrology, Shanghai Pudong Hospital, Fudan University, Pudong Medical Center, 2800 Gongwei Road, Huinan Town, Pudong New Area, Shanghai, China. E-mail: xingjin339662@163.com

References

[1] Liu Y, Li X, Jiang S and Ge Q. Tetramethylpyrazine protects against high glucose-induced vascular smooth muscle cell injury through inhibiting the phosphorylation of JNK, p38MAPK, and ERK. J Int Med Res 2018; 46: 3318-3326.

[2] Kyotani Y, Takasawa S and Yoshizumi M. Proliferative pathways of vascular smooth muscle cells in response to intermittent hypoxia. Int J Mol Sci 2019; 20: 2706.

[3] Hatch E, Morrow D, Liu W, Cahill PA and Redmond EM. Differential effects of alcohol and its metabolite acetaldehyde on vascular smooth muscle cell Notch signaling and growth. Am J Physiol Heart Circ Physiol 2018; 314: H131-H137.

[4] Gurusamy PD, Schafer H, Ramamoorthy S and Wink M. Biologically active recombinant human erythropoietin expressed in hairy root cultures and regenerated plantlets of Nicotiana tabacum L. PLoS One 2017; 12: e0182367.

[5] Natalucci G, Latal B, Koller B, Ruegger C, Sick B, Held L, Bucher HU and Fauchere JC; Swiss EPO Neuroprotection Trial Group. Effect of early prophylactic high-dose recombinant human erythropoietin in very preterm infants on neurodevelopmental outcome at 2 years: a randomized clinical trial. JAMA 2016; 315: 2079-2085.

[6] Merelli A, Ramos AJ, Lazarowski A and Auzmendi J. Convulsive stress mimics brain hypoxia and promotes the P-Glycoprotein (P-gp) and erythropoietin receptor overexpression. recombinant human erythropoietin effect on P-gp activity. Front Neurosci 2019; 13: 750.

[7] Park SL, Won SY, Song JH, Kambe T, Nagao M, Kim WJ and Moon SK. EPO gene expression promotes proliferation, migration and invasion via the p38MAPK/AP-1/MMP-9 pathway by p21WAF1 expression in vascular smooth muscle cells. Cell Signal 2015; 27: 470-478.

[8] Ganju A, Khan S, Hafeez BB, Behrman SW, Yallapu MM, Chauhan SC and Jaggi M. miRNA nanotherapeutics for cancer. Drug Discov Today 2017; 22: 424-432.

[9] Hien TT, Garcia-Vaz E, Stenkula KG, Sjogren J, Nilsson J, Gomez MF and Albinsson S. MicroR-

NA-dependent regulation of KLF4 by glucose in vascular smooth muscle. J Cell Physiol 2018; 233: 7195-7205.
[10] Li M, Liu Q, Lei J, Wang X, Chen X and Ding Y. MiR-362-3p inhibits the proliferation and migration of vascular smooth muscle cells in atherosclerosis by targeting ADAMTS1. Biochem Biophys Res Commun 2017; 493: 270-276.
[11] Tan J, Yang L, Liu C and Yan Z. MicroRNA-26a targets MAPK6 to inhibit smooth muscle cell proliferation and vein graft neointimal hyperplasia. Sci Rep 2017; 7: 46602.
[12] Wu W, Shang YQ, Dai SL, Yi F and Wang XC. MiR-26a regulates vascular smooth muscle cell calcification in vitro through targeting CTGF. Bratisl Lek Listy 2017; 118: 499-503.
[13] Sellars E, Gabra M and Salmena L. The complex landscape of PTEN mRNA regulation. Cold Spring Harb Perspect Med 2019; a036236.
[14] Di Cristofano A and Pandolfi PP. The multiple roles of PTEN in tumor suppression. Cell 2000; 100: 387-390.
[15] Ma SC, Zhang HP, Jiao Y, Wang YH, Zhang H, Yang XL, Yang AN and Jiang YD. Homocysteine-induced proliferation of vascular smooth muscle cells occurs via PTEN hypermethylation and is mitigated by Resveratrol. Mol Med Rep 2018; 17: 5312-5319.
[16] Zhang J, Gao F, Ni T, Lu W, Lin N, Zhang C, Sun Z, Guo H and Chi J. Linc-POU3F3 is overexpressed in in-stent restenosis patients and induces VSMC phenotypic transformation via POU3F3/miR-449a/KLF4 signaling pathway. Am J Transl Res 2019; 11: 4481-4490.
[17] Liu K, Liu C and Zhang Z. lncRNA GAS5 acts as a ceRNA for miR-21 in suppressing PDGF-bb-induced proliferation and migration in vascular smooth muscle cells. J Cell Biochem 2019; 120: 15233-15240.
[18] Guha S, Cullen JP, Morrow D, Colombo A, Lally C, Walls D, Redmond EM and Cahill PA. Glycogen synthase kinase 3 beta positively regulates Notch signaling in vascular smooth muscle cells: role in cell proliferation and survival. Basic Res Cardiol 2011; 106: 773-785.
[19] Samarakoon R and Higgins PJ. The cytoskeletal network regulates expression of the profibrotic genes PAI-1 and CTGF in vascular smooth muscle cells. Adv Pharmacol 2018; 81: 79-94.
[20] Rivera-Cervantes MC, Jarero-Basulto JJ, Murguia-Castillo J, Marin-Lopez AG, Gasca-Martinez Y, Cornelio-Martinez S and Beas-Zarate C. The recombinant human erythropoietin administered in neonatal rats after excitotoxic damage induces molecular changes in the hippocampus. Front Neurosci 2019; 13: 118.
[21] Schiffl H. Correlation of blood pressure in end-stage renal disease with platelet cytosolic free calcium concentration during treatment of renal anemia with recombinant human erythropoietin. Int J Artif Organs 1992; 15: 343-348.
[22] Gogusev J, Zhu DL, Herembert T, Ammarguellat F, Marche P and Drueke T. Effect of erythropoietin on DNA synthesis, proto-oncogene expression and phospholipase C activity in rat vascular smooth muscle cells. Biochem Biophys Res Commun 1994; 199: 977-983.
[23] Zhao Y, Pogue AI and Lukiw WJ. MicroRNA (miRNA) signaling in the human CNS in sporadic Alzheimer's Disease (AD)-novel and unique pathological features. Int J Mol Sci 2015; 16: 30105-30116.
[24] Liu J, Xiao X, Shen Y, Chen L, Xu C, Zhao H, Wu Y, Zhang Q, Zhong J, Tang Z, Liu C, Zhao Q, Zheng Y, Cao R and Zu X. MicroRNA-32 promotes calcification in vascular smooth muscle cells: implications as a novel marker for coronary artery calcification. PLoS One 2017; 12: e0174138.
[25] Chen WJ, Chen YH, Hsu YJ, Lin KH and Yeh YH. MicroRNA-132 targeting PTEN contributes to cilostazol-promoted vascular smooth muscle cell differentiation. Atherosclerosis 2018; 274: 1-7.