European Review for Medical and Pharmacological Sciences 2016; 20: 4167-4175

MicroRNA-142 promotes the expression of eNOS in human peripheral blood-derived endothelial progenitor cells *in vitro*

H.-W. ZHANG[1], H. LI[1], H. YAN[2], B.-L. LIU[2]

[1]Department of Neurosurgery, Second Hospital of Tianjin Medical University, Tianjin China
[2]Department of Neurosurgery, Tianjin Huanhu Hospital, Tianjin, China

Hong-Wei Zhang and Hong Li contributed equally to this work.

Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are small non-coding RNAs (18-25nt) that regulate gene expression mainly through affecting post-transcriptional modification. In this research, the human peripheral blood-derived endothelial progenitor cells were isolated to research the inter-coordination of miR-142, ADAMTS-1, VEGF and eNOS through gene over-expressed in EPCs.

MATERIALS AND METHODS: Endothelial progenitor cells (EPCs) were isolated from human peripheral blood, and demonstrate the interaction of miR-142 and targets (ADAMTS-1) *in vitro*.

RESULTS: The results showed that miR-142 could promote the productivity of NO through inhibited ADAMTS-1 expression (inhibitor of VEGF), activated function of VEGF and eNOS in human EPCs. This finding suggests that miR-142 and eNOS may have cooperative effect in vessel tone and play an important role in the angiogenesis.

CONCLUSIONS: In our research, we demonstrated that the miR-142 can promote eNOS expression through down-regulated ADAMTS-1 expression (inhibitor of VEGF) in human EPCs, these results provide a new insight in microRNA regulation of vessel tone and angiogenesis.

Key Words:
microRNA-142, ADAMTS-1, VEGF, eNOS, NO.

Introduction

Endothelial nitric oxide synthase (eNOS) is a constitutively expressed gene in endothelial cells involved in the production of nitric oxide (NO), which plays a central role in maintaining endothelial cell functional integrity, regulating hemodynamics, and establishing collateral circulation[1]. Adequate NO production, consequent to adequate eNOS expression and activity, is essential for preventing thrombotic and atherogenic processes[2]. It has been shown that vascular endothelial growth factor (VEGF) inhibition induces a decrease in eNOS expression and thus in NO production[3], and that this phenomenon is linked to the induction of hypertension, one of the most common dose-limiting toxicities of VEGF inhibitors[4]. Previous studies have demonstrated an association between specific eNOS polymorphisms and hypertension[5].

Endothelial progenitor cells (EPCs) are precursors of vascular endothelial cells[6]. EPCs originate from bone marrow with similar angioblast and umbilical vein endothelial cells, which together belong to a subgroup of hematopoietic stem cells[7]. There are two sources of EPCs that can be detected *in vitro*, early and late EPCs. While early EPCs display a linear growth structure termed spindle-shaped, late EPCs form cobblestone-like, oval shaped structures[8,9]. EPCs not only take part in vascularization during embryonic development, but also participate in postnatal vascularization and reparative processes post-trauma[10]. Therefore, EPCs hold extensive prospects for vascular tissue engineering and possible clinical application in coronary artery disease and wound healing[8].

A disintegrin and metalloproteinase (ADAM) represent a protein family possessing both metalloproteinase and disintegrin domains. Recently, the ADAM protein family has emerged as a key participant in a diverse array of biological and pathological processes[11,12]. A disintegrin and metalloproteinase with thrombospondinmotif-1 (ADAMTS-1) is a new member protein of the ADAM family, which was originally identified by differential display analysis as a gene highly expressed in the murine colon cachexigenic tumor[13]. However, some research suggested ADAMTS-1 play an

Corresponding Author: Hua Yan, MD; e-mail: hua_yan2008@163.com

inhibitor of VEGF in the progress of physiology and pathology[11,14]. MicroRNA-142 plays an important role in angiogenesis and cell infiltration of cancer, but the regulated mechanism is poorly understood. In this research, the human peripheral blood-derived endothelial progenitor cells were isolated to research the inter-coordination of miR-142, ADAMTS-1, VEGF and eNOS through gene over-expressed in EPCs.

Materials and Methods

Materials and Reagents

Peripheral blood was sampled from healthy volunteers. This study was approved by the Research Ethics Committees of Tianjin Medical University. Informed and written consent was obtained from each volunteer.

Isolation and Culture of Endothelial Progenitor Cells

Peripheral blood-derived mononuclear cells were harvested using 1.077 g/ml Percoll solution (Amersham. Pharmacia Biotech, Uppsala, Sweden). The Percoll solution was slowly added into a centrifuge tube followed by 5 ml of peripheral blood and the tube was centrifuged at 400 g for 20 min. The white nebulous layer was collected and washed twice using DMEM (Gibco, Carlsbad, CA, USA) and centrifuged at 200 ×g for 5 min. The cell suspension containing the majority of the EPCs was obtained using flow cytometry employing an anti-CD34 antibody (Cambridge, MA, USA). Cells were seeded on fibronectin-coated plastic plates in EGM-2MV medium (Lonza, Allendale, NJ USA) and cultured at 37 °C in 5% CO_2. Half of the medium was replaced after 3 days and was then fully replaced once every 3 days. When the cultured cells reached 70–80% confluence, they were removed from culture dishes using 0.25% (w/v) trypsin and subcultured at a 1:3 ratio.

Expression of Genes by Recombinant Adenoviruses

Recombinant adenoviruses were generated by using AdEasy technology[15,16]. The coding regions of ADAMTS-1 was PCR-amplified and cloned into an adenoviral shuttle vector, the pre-miR-142 sequence was synthesized by Sangon Biotech (Shanghai, China), cloned into an adenoviral vector, and then used to recombine adenoviruses in HEK293 cells. The resulting adenoviruses designated as AdVEGF also express GFP as a marker for monitoring infection efficiency. Analogous adenovirus only expressing monomeric GFP (Ad-GFP) was used as a control.

Immunofluorescence

Surface antigen of EPCs was detected by immunofluorescence (IF) staining. The EPCs were fixed in 4% paraformaldehyde (St. Louis, MO, USA) for 20 min, then blocked for 15 min with methanol containing 0.1% Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA), and then incubated in a goat serum working solution for 30 min to block nonspecific binding. The EPCs were then incubated at room temperature for 1 h with primary antibodies including CD34, CD133 and KDR (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The EPCs were incubated with secondary antibodies conjugated with FITC (goat anti-mouse IgG and goat anti-rat IgG, Boster, Beijing, China). For negative control, phosphate buffered saline (PBS) was used to replace the primary antibodies. Finally, nuclei were labeled by incubation with 4,6 diamidino-2-phenylindole (DAPI)(Sigma-Aldrich, St. Louis, MO, USA). The cells were examined by a phase contrast fluorescence microscope (Olympus, Tokyo, Japan).

RNA Isolation and PCR Analysis

RNA was extracted from EPCs using a Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was reverse transcribed, followed by 30 PCR cycles using RNA PCR kit ver 3.0 (Takara, Dalian, China). Information of gene-specific primer pairs was listed in Table I. PCR was performed in 50 µl of mixture containing 10 µl of 5×PCR Buffer (Takara, Dalian, China), 28.5 µl of ddH_2O, 0.25 µl of Ex-Taq (Takara, Dalian, China), 0.5µl of forward and reverse primers, and 1.5 µl of template cDNA. The cycling conditions consisted of one initial 2-min cycle at 94 °C, followed by 30 30-s cycles at 94 °C (denaturation), one 30-sec cycle at 50-60 °C (annealing), and one 2-min cycle at 72 °C (extension). PCR products were detected by 2.5% agarose gel electrophoresis. Real-time PCR was performed in a 20 µl mixture containing 10 µl SYBR Premix Ex Taq buffer (Takara, Dalian, China), 0.4 µl ROX Reference Dye, 0.8 µM each of forward and reverse primers (Table I), 1 µl template cDNA and 7 µl ddH2O. The cycling conditions consisted of initial 10 sec at 95°C followed by 40 cycles of two-temperature cycling: 5 sec at 95 °C (for denaturation) and 34 sec at 60 °C (for annealing and polymerization). Each expe-

Table I. Comparison of intra-operative and postoperative bleeding volume (ml).

Gene	Primer Sequence
ADAMTS-1	F 5 TCTTCCTTGACGGTGCTTCC 3' R 5 TCTTCCTTGACGGTGCTTCC 3
VEGF	F 5' ACGGTCCCTCTTGGAATTGG 3' R 5' CGGCCGCGGTGTGTCTA 3'
eNOS	F 5' TCCGGAAGGCTTTTGATCCC 3' R 5' AAACGGACACTAAGGCAGCA 3'
VEGFR2	F 5' CGGTCAACAAAGTCGGGAGA 3' R 5 CAGTGCACCACAAAGACACG' 3'
PI3K	TCTCCTCCTCCTCCTGCTTC TCTCCTCCTCCTCCTGCTTC
GAPDH	GCTCCCTCTTTCTTTGCAGC CCATGAGTCCTTCCACGATACC
AKT	TCTCCTCCTCCTCCTGCTTC TCTCCTCCTCCTCCTGCTTC
miR142	GATATTTAATGTACATAAATAT

riment was performed in duplicates in a 96-well plate and repeated three times. Gene expression was detected on an ABI 7500 real-time PCR system (ABI, Foster City, CA, USA). miRNAs were isolated from cells using microRNA isolation kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. cDNA synthesis was carried out with the High Capacity cDNA synthesis kit (Applied Biosystems, Foster City, CA, USA) using 2 ng of RNA as a template. The miRNA sequence-specific reverse transcription PCR primers for miR-124 and endogenous control U6 were purchased from Ambion (Austin, TX, USA). Real-time PCR analysis was carried out using Applied Biosystems 7500 real-time PCR system. The gene expression threshold cycle (CT) values of miRNAs from each sample were calculated by normalizing with internal control U6 and relative quantitation values were plotted. The expression level was calculated by the $2^{-\Delta\Delta Ct}$ method to compare the relative expression.

Western Blot

The cells were lysed for total protein extraction. The protein concentration was determined by the bicinchoninic acid (BCA) method (Beyotime, Beijing, China), and 15 μg of protein lysates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The electrophoresed proteins were transferred to 0.2

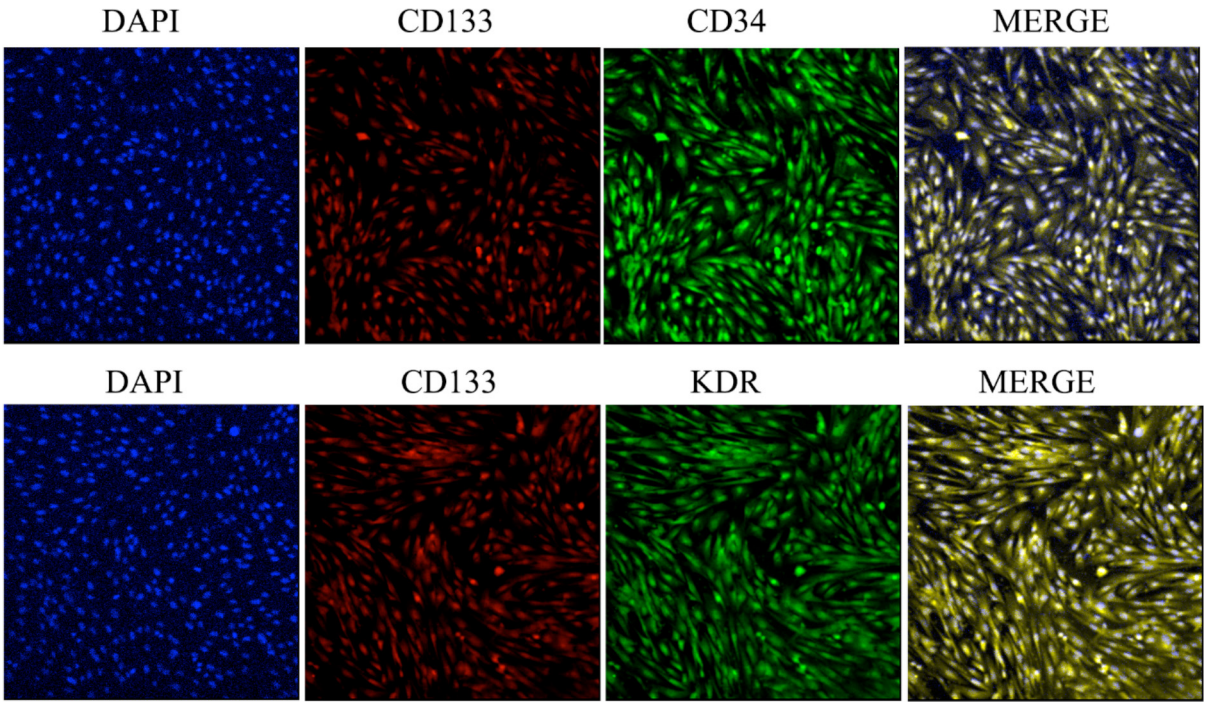

Figure 1. Special gene characteristics of human peripheral blood-derived EPCs at different passages. Immunofluorescence staining results showed that human peripheral blood-derived EPCs were positive for the CD34, CD133, and KDR. (Magnification 100X).

μm polyvinylidene fluoride) (PVDF) membranes (Millipore, St Charles, MO, USA), which were blocked in 5% non-fat milk and incubated overnight at 4 °C with diluted antibodies against VEGF (1:1000, Cell Signaling Technology, Danvers, MA, USA) , ADAMTS-1 (1:1000, Cell Signaling Technology, USA) and eNOS (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membranes were then incubated with the HRP-conjugated secondary antibody (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA, USA). After 3 items of washings with phosphate buffer solution with Tween-20 (PBST), the membranes were probed using ultra-enhanced chemiluminescence Western blotting detection reagents. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control.

Statistical Analysis

Statistical analyses of the data were performed with a one-way ANOVA followed by the Tukey-Kramer honestly significant difference (HSD) test for the three sets of results. A p-value of less than 0.05 was considered significant. Statistical analyses were done with a JMP Statistical Discovery Software (SAS Institute, Cary, NC).

Results

Special Genes Expression in Human Peripheral Blood-Derived EPCs

The isolated peripheral blood-derived mononuclear cells were round or irregular, which began to adhere and grow after 48 h. The cells proliferated quickly, displaying obviously karyokinesis and different morphology including fusiform, triangular or irregular shape after 5 days. CD133, CD34 and KDR are the specific markers of EPCs. In this research, we examined the expression of these specific markers in human peripheral blood-derived EPCs and found that the cells expressed these three makers.

The interaction of miR-142 and ADAMTS-1 in Human Peripheral Blood-Derived EPCs

miRNA target genes are likely to have relatively long and conserved 3′UTR [17]. We noticed that ADAMTS-1 has a long evolutionarily conserved 3′UTR, so we used the TargetScan algorithm [18] to search for miRNAs that could potentially regulate ADAMTS-1. The miR-142 has the same putative target binding sites in ADAMTS-1 in the human genome (Figure 2). So, we over-expressed miR-142 in EPCs and observed the protein level of the target gene. The resulting adenoviruses designated as Ad-pre-miR-142/ Ad-ADAMTS-1 also express GFP as a marker for monitoring infection efficiency. Expression of GFP was observed after transfected 72h using fluorescence microscope. The results demonstrated that the GFP were positive in EPCs. To determine the expression of miR-142 in human peripheral blood-derived EPCs, we detected the mRNA level of miR-142 in EPCs after transfection using Real-time PCR. And to determine the expression of ADAMTS-1 in EPCs, we detected the protein level of ADAMTS-1 in EPCs after transfection using Western blotting, as shown in Figure 2, mRNA level of miR-142 and protein levels of ADAMTS-1 were significantly elevated respectively after transfection. Protein expression of ADAMTS-1 was performed on ADAMTS-1 transfected cells according to Image J tools comparative method (Figure 2).

The Expression of Downstream Genes After Over-Expressed miR-142 and ADAMTS-1 in Human Peripheral Blood-Derived EPCs

Real time PCR was used to detect the gene expression of downstream genes, including VEGFR2, PI3K, Akt and eNOS. The PI3K signaling pathway is one of the major signalling pathways that have been identified as important in the production of eNOS. The result demonstrated that after transfected miR-142, the specific downstream genes, including VEGFR2, PI3K, Akt, and eNOS, were expressed and gene expression level showed a time-lapse increase in human peripheral blood-derived EPCs, but after transfected ADAMTS-1, the level of downstream genes showed a time-lapse decrease in EPCs (Figure 3).

Level of NO After Transfected miR-142 and ADAMTS-1 in Human Peripheral Blood-Derived EPCs

In mammals including humans, NO is an important cellular signaling molecule involved in many physiological and pathological processes. eNOS is primarily responsible for the generation of NO in the vascular endothelium, a monolayer of flat cells lining the interior surface of blood vessels, at the interface between circulating blood in the lumen and the remainder of the vessel wall. The level of eNOS was significantly elevated after transfected miR-142 and, then, the concentration of NO will be tested using

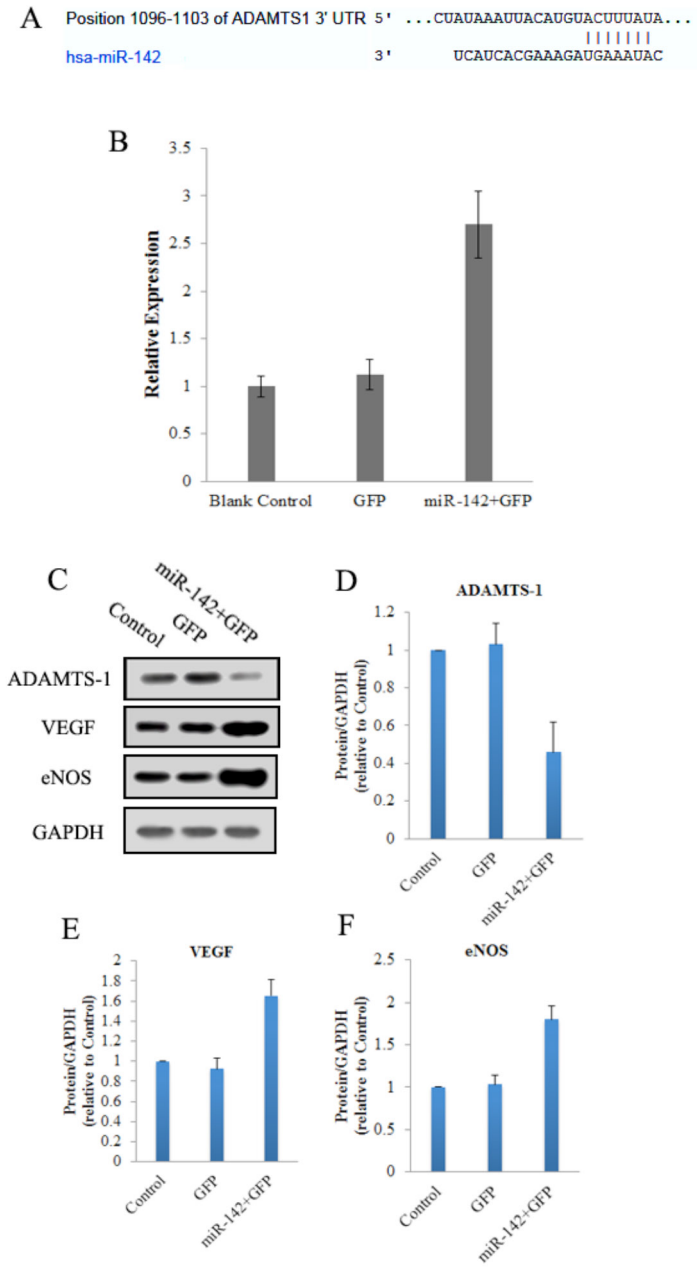

Figure 2. The target site of miRNA-142 and up-regulation of miR-142 in pre-miR-142 transfected human peripheral blood-derived EPCs. ***A,*** The miR-142 complementary sites with 3'UTR of ADAMTS-1. ***B,*** Human peripheral blood-derived EPCs were transfected with adenoviruses as described in the Methods, and the expression of miR-142 was quantified by Real-time PCR. ***C,*** Effect of miR-142 on protein level of ADAMTS-1, VEGF, and eNOS. D, E and F Quantification of ADAMTS-1, VEGF and eNOS in human peripheral blood-derived EPCs transfected with miR-142, GFP or Control for 72 h.

a spectrophotometer. The concentration of NO in cell culture medium were detected after one week of miR-142 and ADAMTS-1 transfection respectively using a spectrophotometer. The results demonstrated that concentration of NO had significant difference after transfected miR-142.

Discussion

NO is a gaseous molecule with an astonishingly wide range of physiological and pathophysiological activities, including the regulation of vessel tone and angiogenesis in wound healing, inflammation, ischemic cardiovascular diseases and malignant diseases. Recent data[19] have revealed the predominant role of endothelial nitric oxide synthase (eNOS), an endothelial-cell-specific isoform of NO producing enzyme, in both angiogenesis (the development of new blood vessels derived from existing vessels) and vasculogenesis (blood vessel formation *de novo* from progenitor cells). Also, successes in gene therapy, together with the recent development of an eNOS-speci-

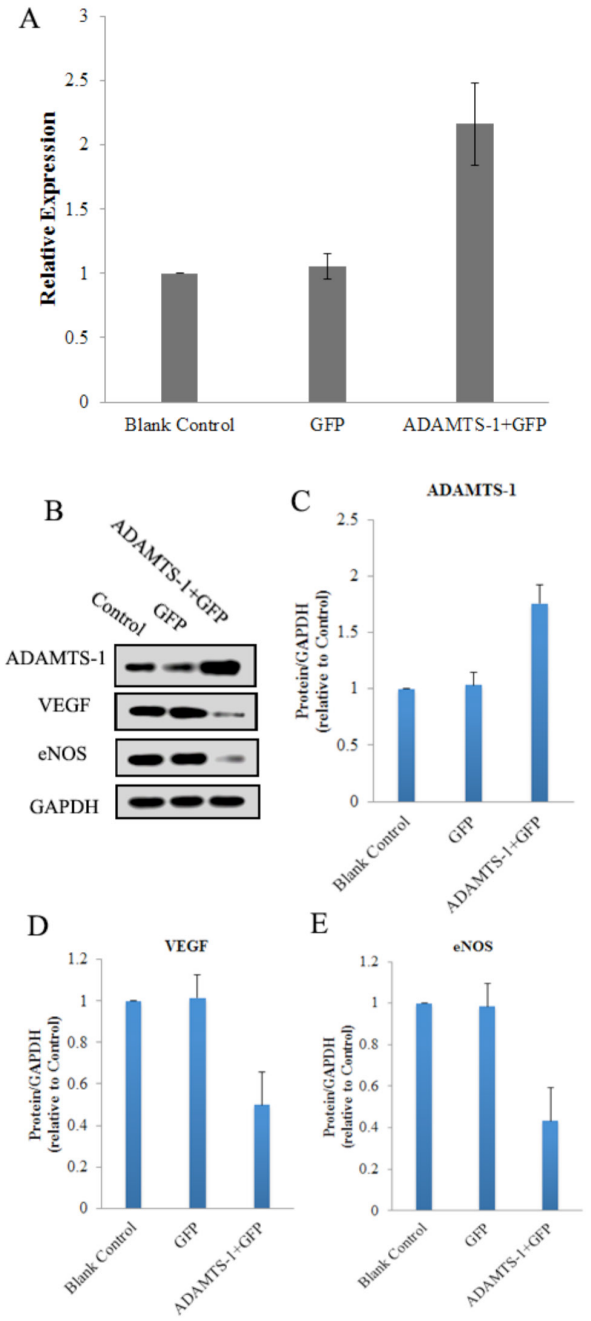

Figure 3. Up-regulation of ADAMTS-1(target of miR-142) in transfected human peripheral blood-derived EPCs. ***A,*** Human peripheral blood-derived EPCs were transfected with adenoviruses as described in the Methods, and the expression of ADAMTS-1 mRNA was quantified by Real-time PCR. B, Transfected effect on protein level of ADAMTS-1, VEGF and eNOS. ***C, D*** and ***E,*** Quantification of ADAMTS-1, VEGF and eNOS in human peripheral blood-derived EPCs transfected with ADAMTS-1, GFP or Control for 72 h.

fic inhibitor, suggest that the modulation of eNOS might be a potent new strategy for the control of pathological neovascularization.

MicroRNAs are small non-coding RNA that exhibit a high degree conservation of structure and function in plant and animal. They exist in two forms of pre-miRNAs and mature miRNAs, and only the mature miRNAs-mediated by the two RNase III endonucleases Dicer and Drosha[20] play a key biological role. The mature miRNAs inhibit protein translation by binding the 3'-UTR of target mRNA partly, while they induce target mRNA cleavage through binding mRNA with perfect complementarity[21]. A target gene analysis of miRNAs may lead to better understanding of the mechanisms by which miRNAs mediate proliferation and apoptosis in cells. ADAMTS-1 is a soluble matrix metalloprotease molecule that inhibits angiogenesis by mechanisms that may relate to direct sequestration of VEGF or by activating the release of anti-angiogenic thrombospondin-derived peptides[22]. VEGF is an endothelial-specific growth factor that induces angiogenesis, i.e., the sprouting of capillaries from preexisting vessels in vivo. eNOS is an essential molecule in mediating VEGF -induced angiogenesis and endothelial function via production of NO. Moreover, the protein level of eNOS is up-regulated in response to VEGF.

The miR-142 has previously been shown to be expressed in murine BM, spleen, and thymus and was especially high in B cells and T-cell subsets [23].Recent studies have reported that miR-142, which is located at chromosome 17q22, is important in the tumorigenesis of hepatocellular carcinoma and esophageal squamous cell carcinoma[24]. However, the function of miR-142 in endothelial cells is largely unknown. In this research, targets of miR-142 were analyzed using TargetScan tools; ADAMTS-1 were selected and demonstrated which was regulated by miR-142 via complementary sequence in 3'UTR. This region is highly homologous between human and mouse, suggesting an evolutionarily conserved regulatory mechanism for controlling miR-142 expression. A recent study provided some evidence that miR-142 overexpression in hepatocellular carcinoma cells exerts an anti-tumor effect, which is likely related to the capacity of this microRNA to target the 3′UTR region of ADAMTS-1 mRNA[24]. In our research, we demonstrated that the miR-142 can promote eNOS expression through down-regulated ADAMTS-1 expression (inhibitor of VEGF) in human EPCs, these results provide a new insight in microRNA regulation of vessel tone and angiogenesis.

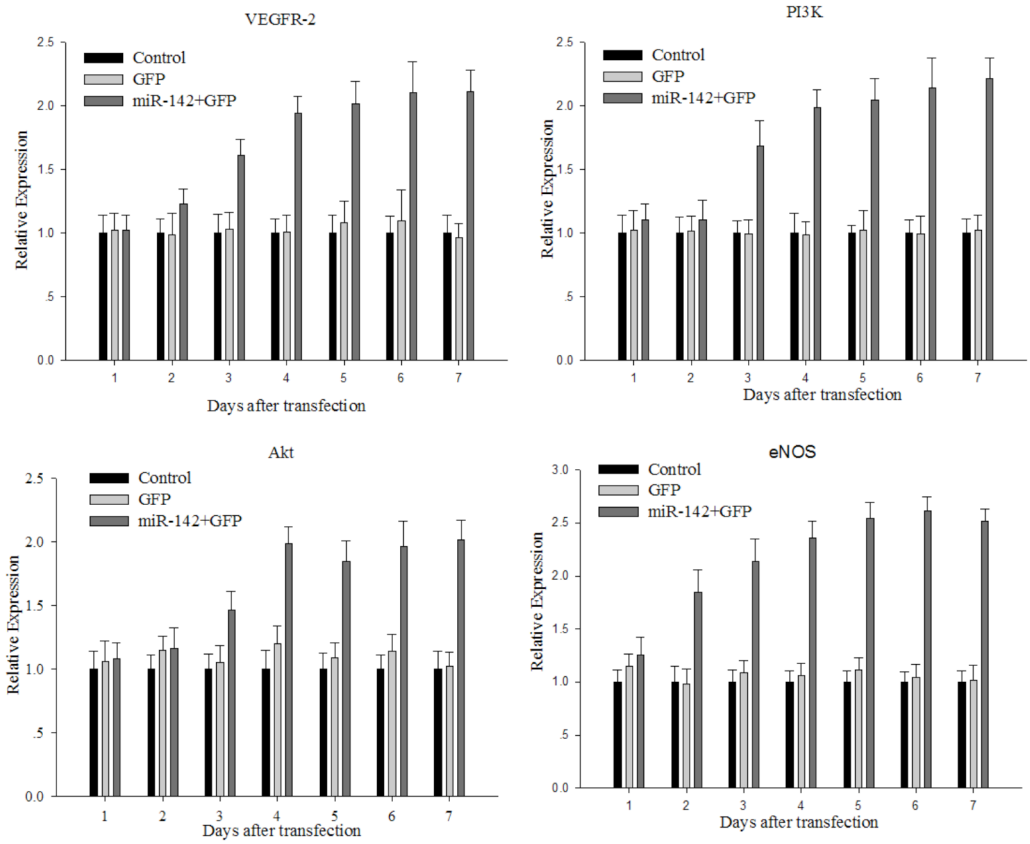

Figure 4. Gene expression of PI3K signaling pathway detected in continuously cultured human peripheral blood-derived EPCs. VEGFR-2 is a special marker for the endothelial cell line, and it is a mechanotransducer for flow-mediated PI3K-Akt-eNOS activation and NO-dependent vasodilation in vivo. Phosphoinositide 3-kinase (PI3K) and its downstream serine/threonine protein kinase Akt (protein kinase B) mediate phosphorylation of eNOS at Ser1179 (based on the bovine eNOS sequence and equivalent to human eNOS-Ser1177). Expression of VEGFR-2, PI3K, Akt and eNOS were no significantly changed in the normal human peripheral blood-derived EPCs. However, following overexpression of miR-142, expression increased over time.

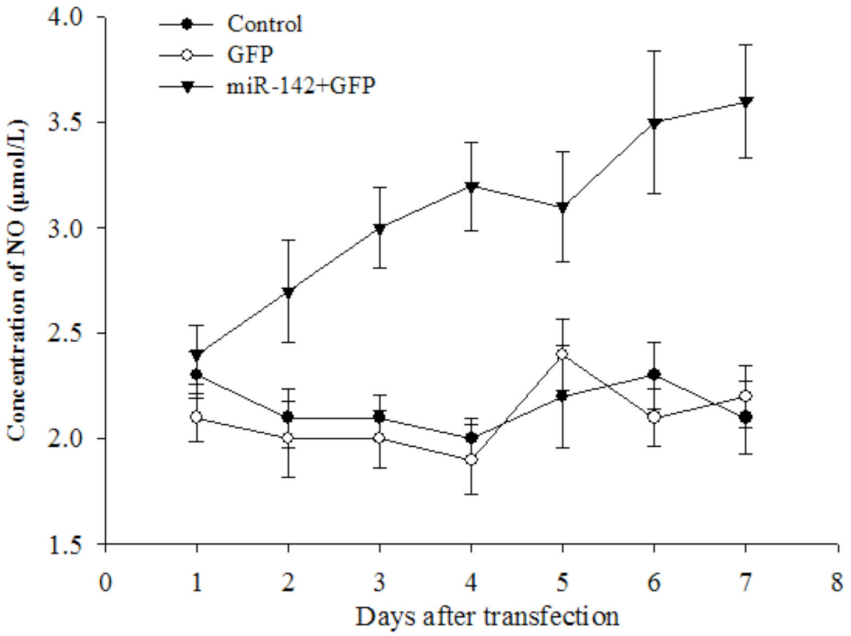

Figure 5. The concentration of NO after transfected miR-142 in human peripheral blood-derived EPCs. The concentration of NO was detected using a spectrophotometer. The results demonstrated that the concentration of NO had a significant difference after transfected miR-142.

Conclusions

In this study, we analyzed the role of miR-142 in the eNOS expression of human EPCs. The results demonstrated that miR-142 could promote the productivity of NO through inhibited ADAMTS-1 expression (inhibitor of VEGF), the activated function of VEGF and eNOS in human EPCs. This finding suggests that miR-142 and eNOS may have cooperative effect in vessel tone and play an important role in the angiogenesis.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. N Engl J Med 1994; 330: 1431-1438.
2) Oemar BS, Tschudi MR, Godoy N, Brovkovich V, Malinski T, Luscher TF. Reduced endothelial nitric oxide synthase expression and production in human atherosclerosis. Circulation 1998;97: 2494-2498,Muluk NB, Arikan OK, Atasoy P, Kilic R, Yalcinozan ET. The role of endothelial nitric oxide synthase (eNOS) in the pathogenesis of sinonasal polyps. Eur Rev Med Pharmacol Sci 2014; 18: 918-929.
3) Winnik S, Lohmann C, Siciliani G, von Lukowicz T, Kuschnerus K, Kraenkel N, Brokopp CE, Enseleit F, Michels S, Ruschitzka F, Luscher TF, Matter CM. Systemic VEGF inhibition accelerates experimental atherosclerosis and disrupts endothelial homeostasis--implications for cardiovascular safety. Int J Cardiol 2013; 168: 2453-2461.
4) Facemire CS, Nixon AB, Griffiths R, Hurwitz H, Coffman TM. Vascular endothelial growth factor receptor 2 controls blood pressure by regulating nitric oxide synthase expression. Hypertension 2009; 54: 652-658.
5) Eechoute K, van der Veldt AA, Oosting S, Kappers MH, Wessels JA, Gelderblom H, Guchelaar HJ, Reyners AK, van Herpen CM, Haanen JB, Mathijssen RH, Boven E. Polymorphisms in endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) predict sunitinib-induced hypertension. Clin Pharmacol Ther 2012; 92: 503-510.
6) Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, Witzenbichler B, Schatteman G, Isner JM. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997; 275: 964-967.
7) Spadaccio C, Pollari F, Casacalenda A, Alfano G, Genovese J, Covino E, Chello M. Atorvastatin increases the number of endothelial progenitor cells after cardiac surgery: a randomized control study. J Cardiovasc Pharmacol 2010; 55: 30-38.
8) Gulati R, Jevremovic D, Peterson TE, Chatterjee S, Shah V, Vile RG, Simari RD. Diverse origin and function of cells with endothelial phenotype obtained from adult human blood. Circ Res 2003; 93: 1023-1025.
9) Bai C, Hou L, Li F, He X, Zhang M, Guan W. Isolation and biological characteristics of beijing Fatty chicken skeletal muscle satellite cells. Cell Commun Adhes 2012; 19: 69-77.
10) Bonello L, Basire A, Sabatier F, Paganelli F, Dignat-George F. Endothelial injury induced by coronary angioplasty triggers mobilization of endothelial progenitor cells in patients with stable coronary artery disease. J Thromb Haemost 2006; 4: 979-981.
11) Shindo T, Kurihara H, Kuno K, Yokoyama H, Wada T, Kurihara Y, Imai T, Wang Y, Ogata M, Nishimatsu H, Moriyama N, Oh-hashi Y, Morita H, Ishikawa T, Nagai R, Yazaki Y, Matsushima K. ADAMTS-1: a metalloproteinase-disintegrin essential for normal growth, fertility, and organ morphology and function. J Clin Invest 2000; 105: 1345-1352.
12) Wolfsberg TG, Primakoff P, Myles DG, White JM. ADAM, a novel family of membrane proteins containing A Disintegrin And Metalloprotease Domain: multipotential functions in cell-cell and cell-matrix interactions. J Cell Biol 1995; 131: 275-278.
13) Kuno K, Kanada N, Nakashima E, Fujiki F, Ichimura F, Matsushima K. Molecular cloning of a gene encoding a new type of metalloproteinase-disintegrin family protein with thrombospondin motifs as an inflammation associated gene. J Biol Chem 1997; 272: 556-562.
14) Mathsyaraja H, Thies K, Taffany DA, Deighan C, Liu T, Yu L, Fernandez SA, Shapiro C, Otero J, Timmers C, Lustberg MB, Chalmers J, Leone G, Ostrowski MC. CSF1-ETS2-induced microRNA in myeloid cells promote metastatic tumor growth. Oncogene 2015;34: 3651-3661,Vazquez F, Hastings G, Ortega MA, Lane TF, Oikemus S, Lombardo M, Iruela-Arispe ML. METH-1, a human ortholog of ADAMTS-1, and METH-2 are members of a new family of proteins with angio-inhibitory activity. J Biol Chem 1999; 274: 23349-23357.
15) Kang Q, Sun MH, Cheng H, Peng Y, Montag AG, Deyrup AT, Jiang W, Luu HH, Luo J, Szatkowski JP, Vanichakarn P, Park JY, Li Y, Haydon RC, He TC. Characterization of the distinct orthotopic bone-forming activity of 14 BMPs using recombinant adenovirus-mediated gene delivery. Gene Ther 2004; 11: 1312-1320.
16) Cheng H, Jiang W, Phillips FM, Haydon RC, Peng Y, Zhou L, Luu HH, An N, Breyer B, Vanichakarn P, Szatkowski JP, Park JY, He TC. Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs). J Bone Joint Surg Am 2003; 85-A: 1544-1552.
17) Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136: 215-233.
18) Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that

thousands of human genes are microRNA targets. Cell 2005; 120: 15-20.

19) Duda DG, Fukumura D, Jain RK. Role of eNOS in neovascularization: NO for endothelial progenitor cells. Trends Mol Med 2004; 10: 143-145.

20) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

21) 5 MicroRNA function: multiple mechanisms for a tiny RNA? RNA 2005; 11: 1753-1761,Zamore PD, Haley B. Ribo-gnome: the big world of small RNAs. Science 2005;309: 1519-1524,Bai C, Li X, Gao Y, Wang K, Fan Y, Zhang S, Ma Y, Guan W. Role of microRNA-21 in the formation of insulin-producing cells from pancreatic progenitor cells. Biochim Biophys Acta 2016; 1859: 280-293.

22) Lee NV, Sato M, Annis DS, Loo JA, Wu L, Mosher DF, Iruela-Arispe ML. ADAMTS1 mediates the release of antiangiogenic polypeptides from TSP1 and 2. EMBO J 2006;25: 5270-5283,Luque A, Carpizo DR, Iruela-Arispe ML. ADAMTS1/METH1 inhibits endothelial cell proliferation by direct binding and sequestration of VEGF165. J Biol Chem 2003; 278: 23656-23665.

23) Lv M, Zhang X, Jia H, Li D, Zhang B, Zhang H, Hong M, Jiang T, Jiang Q, Lu J, Huang X, Huang B. An oncogenic role of miR-142-3p in human T-cell acute lymphoblastic leukemia (T-ALL) by targeting glucocorticoid receptor-alpha and cAMP/PKA pathways. Leukemia 2012; 26: 769-777.

24) Wu L, Cai C, Wang X, Liu M, Li X, Tang H. MicroRNA-142-3p, a new regulator of RAC1, suppresses the migration and invasion of hepatocellular carcinoma cells. FEBS Lett 2011; 585: 1322-1330.