Int J Clin Exp Pathol 2015;8(1):414-422
www.ijcep.com /ISSN:1936-2625/IJCEP0003404

Original Article

MiR-21 suppresses endothelial progenitor cell proliferation by activating the TGFβ signaling pathway via downregulation of WWP1

Keqiang Zuo[1*], Maoquan Li[1*], Xiaoping Zhang[1], Chenghui Lu[1], Shi Wang[1], Kangkang Zhi[2], Bin He[3]

[1]Department of Interventional & Vascular Surgery, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai 200072, China; [2]Department of Vascular and Endovascular Surgery, Changzheng Hospital, Shanghai 200003, China; [3]Department of Anesthesiology and SICU, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Kongjiang Road 1665, Shanghai 200092, China. [*]Equal contributors.

Received October 27, 2014; Accepted December 22, 2014; Epub January 1, 2015; Published January 15, 2015

Abstract: Endothelial damage is strongly associated with cardiovascular diseases such as atherosclerosis. Bone marrow-derived endothelial progenitor cells (EPCs) play an important role in the maintenance of endothelial homeostasis and contribute to re-endothelialization of injured vessels as well as revascularization of ischemic tissues. MicroRNAs (miRNAs) have been reported to regulate EPC biological functions. In this study, we found that EPCs of atherosclerosis patients and EPCs exposed to hypoxia have increased expression of miRNA-21 (miR-21) as well as diminished ability to proliferate. MiR-21 knockdown rescued hypoxia-induced growth arrest in EPCs. Next, we used a luciferase reporter assay to demonstrate that miR-21 downregulates the expression of WW domain-containing protein 1 (WWP1), a negative regulator of TGFβ signaling, by directly targeting the 3'-UTR of WWP1. Finally, miR-21 overexpression or WWP1 knockdown in EPCs significantly activates the TGFβ signaling pathway and inhibits cell proliferation. Taken together, our results indicate that miR-21 suppresses EPC proliferation by activating the TGFβ signaling pathway via downregulation of WWP1. These findings may help the development of strategies to enhance the vitality of EPCs for therapeutic applications.

Keywords: Atherosclerosis, endothelial progenitor cells (EPCs), microRNA-21 (MiR-21), WWP1, TGFβ signaling pathway

Introduction

Endothelial damage is strongly associated with cardiovascular diseases such as atherosclerosis, thrombosis, and hypertension [1]. Bone marrow-derived endothelial progenitor cells (EPCs) play an important role in the maintenance of endothelial integrity and homeostasis, and contribute to re-endothelialization of injured vessels as well as revascularization of ischemic tissues [2]. The number of EPCs is inversely associated with cardiovascular risk factors and vascular function, and independently predicts clinical outcomes of cardiovascular diseases [3]. The therapeutic potential of EPC transplantation in rescue of tissue ischemia has been demonstrated in a few small-scale trials; however, the relative scarcity of circulating EPCs limits the ability to expand these cells in sufficient numbers for some therapeutic applications [4, 5]. Therefore, the development of strategies to enhance proliferation and improve the function of EPCs is key to the success of EPC therapy.

Transforming growth factor-β (TGFβ) is a multifunctional cytokine that controls growth, differentiation, and other functions in most cells. Activation of TGFβ type I receptor leads to phosphorylation of Smads 2 and 3, which, together with Smad4, are translocated into the nucleus to regulate gene transcription. The inhibitory Smad (Smad7) negatively regulates TGFβ signaling by binding to activated type I receptor to cause its ubiquitination and degradation [6]. TGFβ is highly expressed in human atherosclerotic and vascular restenosis lesions [7, 8]. Experimental results indicate that TGFβ exerts both protective and harmful effects on the cardiovascular system through its functional regu-

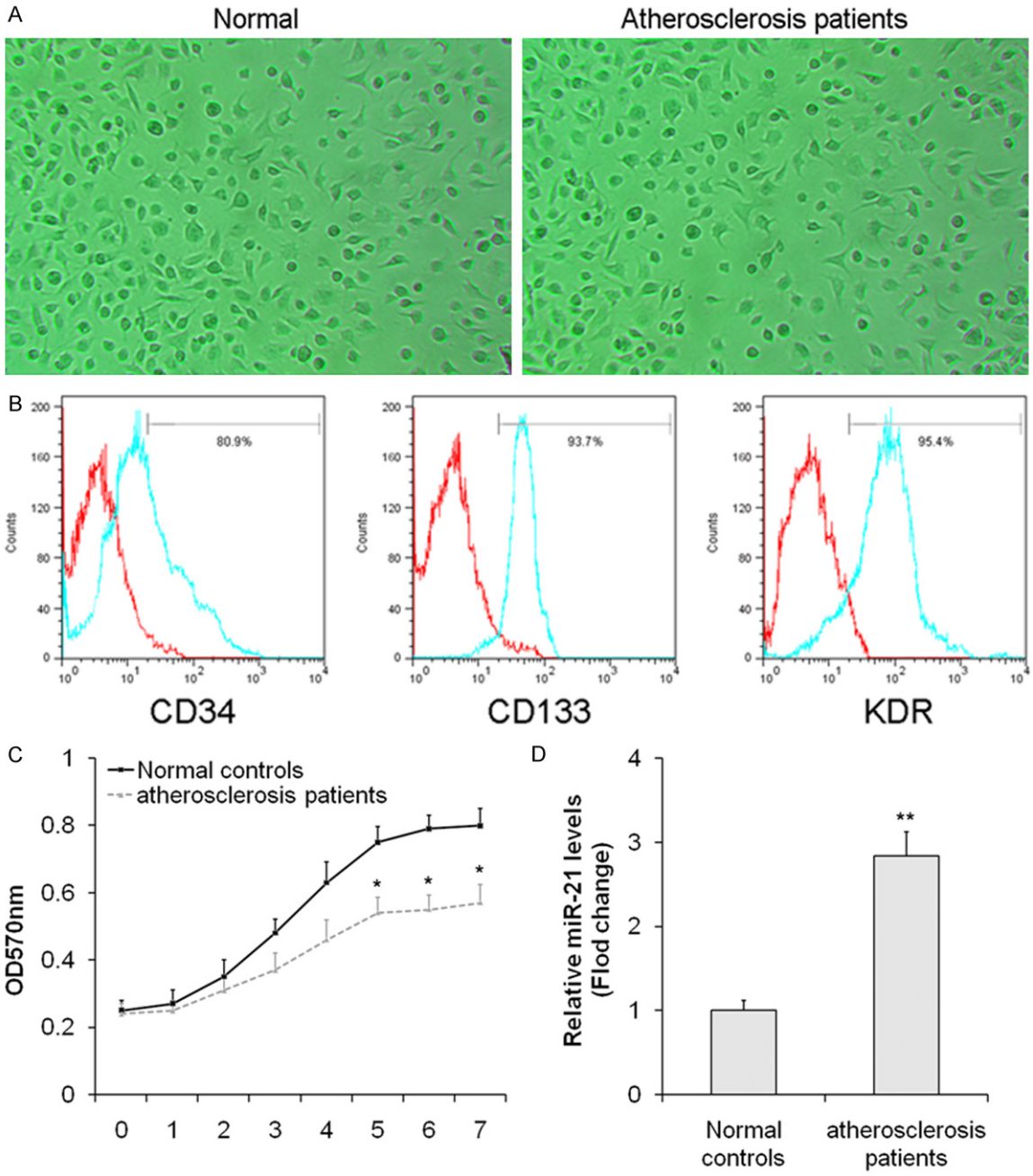

Figure 1. MiR-21 is upregulated in EPCs of atherosclerosis patients. A. EPCs under a phase-contrast microscope (100 ×). B. Expression of stem cell markers CD34 and CD133 and the endothelial lineage marker KDR by flow cytometric analysis. C. Cell proliferation by MTT assay (n = 3). D. MiR-21 expression by quantitative real-time PCR (n = 3). *$P < 0.05$, **$P < 0.01$ vs. normal controls.

lation of endothelial cells, vascular smooth muscle cells, macrophages, and T lymphocytes [9]. A few recent studies suggest that TGFβ also regulates the function of circulating EPCs. In EPC-seeded tissue engineering scaffolds, TGFβ1 promotes endothelial to mesenchymal cell type transformation as well as extracellular matrix production and tissue formation [10]. In isolated EPCs in culture, TGFβ1 antagonizes pioglitazone-induced cell adhesion and differentiation [11]. However, the effects of TGFβ on the proliferation of circulating EPCs are largely unclear.

WW domain-containing protein 1 (WWP1), an E3 ubiquitin ligase, interacts with Smad7 and enhances the binding of Smad7 to TGFβ type I receptor. Thus WWP1 inhibits TGFβ signaling in

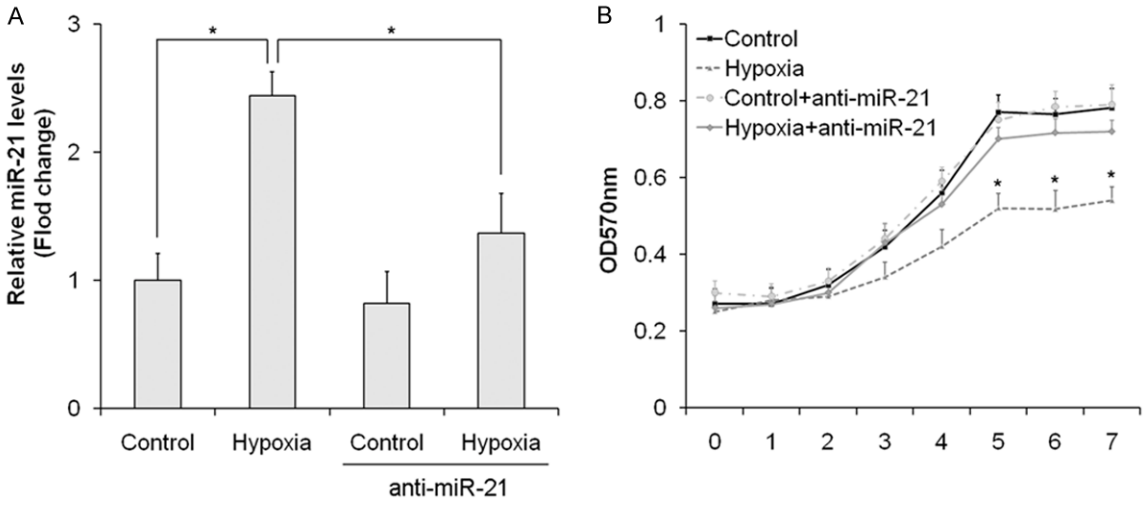

Figure 2. Sequestration of miR-21 attenuates hypoxia-induced growth inhibition of EPCs. Rat EPCs were transfected with 20 nM anti-miR-control or anti-miR-21 and cultured under normoxic (20% O_2, control) or hypoxic (1% O_2, hypoxia) conditions. (A) MiR-21 expression by quantitative real-time PCR (n = 3), $*P < 0.05$. (B) Cell proliferation by MTT assay (n = 3), $*P < 0.05$ vs. all other groups.

cooperation with Smad7 [12]. WWP1 is a putative target of microRNA-21 (miR-21) [13], an miRNA overexpressed in many cancers and associated with tumor growth and metastasis [5]. Studies have indicated cell type-specific effects of miR-21 on neovascularization. While miR-21 expression in cancer cells promotes tumor-associated angiogenesis *in vivo* [14, 15], miR-21 expression in endothelial cells reduces endothelial cell proliferation, migration, and *in vitro* angiogenesis [16]. Interestingly, a recent study links miR-21 to aging-associated senescence in EPCs [17]. MiR-21 overexpression in young EPCs causes cell senescence while miR-21 silencing rejuvenates EPCs and improves EPC angiogenesis *in vitro* and *in vivo* [17].

In this study, we examined miR-21 expression in EPCs of atherosclerosis patients and EPCs exposed to hypoxia. We then investigated the regulatory relationship between miR-21 and WWP1. Finally, we studied the role of miR-21, TGFβ signaling, and their interplay in the regulation of EPC proliferation. Our results may help the development of new strategies to enhance EPC vitality for therapeutic applications.

Materials and methods

Patients

Blood samples were collected from atherosclerosis patients admitted to Shanghai Tenth People's Hospital and healthy volunteers. All study protocols were approved by the Research Ethics Board of the Shanghai Tenth People's Hospital. All patients and healthy volunteers gave informed consent.

Isolation of EPCs

Human and rat EPCs were isolated as previously described [18]. Briefly, human EPCs were isolated using a Becton-Dickinson FACS Aria cytometer (BD Biosciences, America). Rat EPCs were obtained from rat bone marrow mononuclear cells (BMMCs) as follows: Rat BMMCs were isolated from bone marrow cell suspension by density gradient centrifugation utilizing Ficoll-Isopaque (Sigma, USA) according to the manufacturer's instructions. The BMMCs obtained were seeded in six-well plates and cultured in M199 medium at 37°C, 5% CO_2 in a humidified incubator for 1 h. Non-adherent cells were collected and cultured in M199 medium supplemented with 10 ng/ml vascular endothelial growth factor (VEGF, PeproTech, USA), 2 ng/ml basic fibroblast growth factor (bFGF, PeproTech), and 10% fetal bovine serum (FBS, Hyclone, USA) at 37°C, 5% CO_2 for 3 days. Nonadherent cells were removed by washing with phosphate-buffered saline (PBS), and adherent cells were collected and cultured in fresh supplemented M199 medium for 7 days. The identity of EPCs was confirmed by the expression of stem cell markers CD34 and

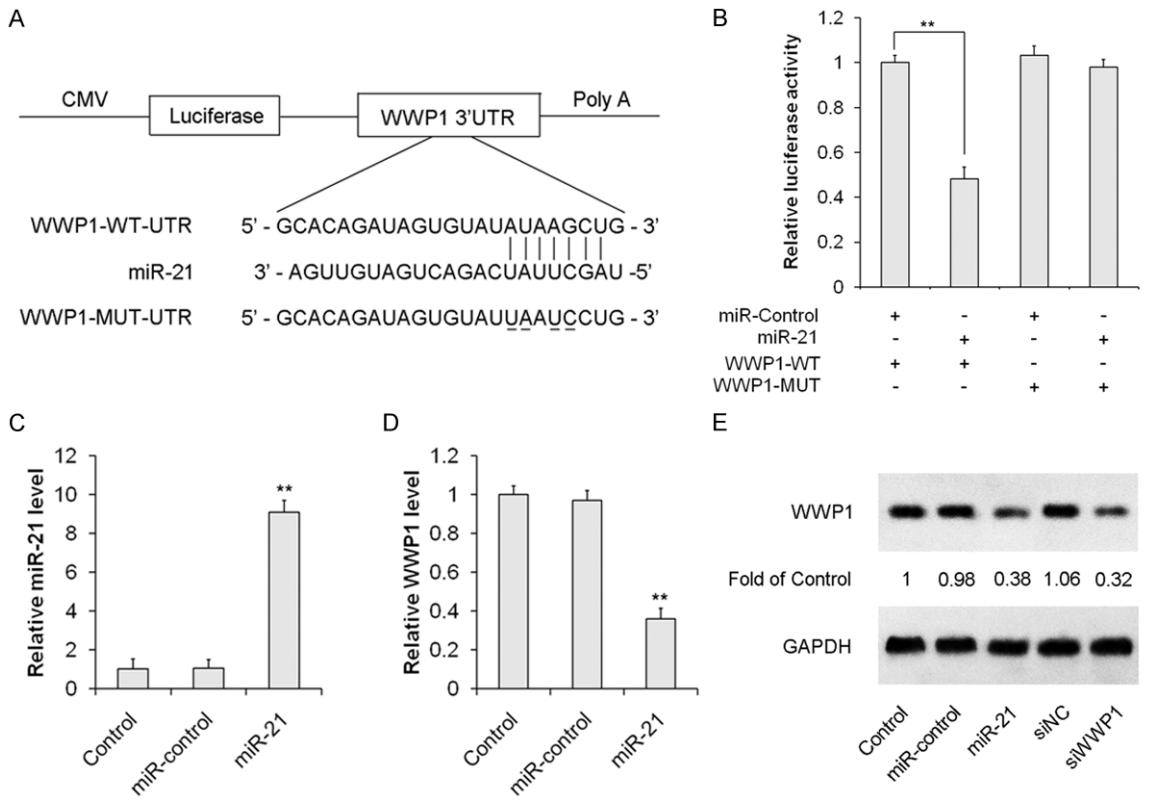

Figure 3. MiR-21 negatively regulates WWP1 expression in EPCs by directly targeting the 3'-UTR of WWP1 gene. A. The luciferase reporter construct containing the wild type or mutant 3'-UTR of WWP1. WWP1-WT-UTR, sequence of the putative miR-21 binding site; WWP1-MUT-UTR, sequence of the mutant miR-21 binding site. B. Luciferase reporter activity in rat EPCs 2 days post transfection (n = 3). C. MiR-21 expression in rat EPCs (control) and rat EPCs transfected with miR-21 or miR-control (n = 3). D. WWP1 mRNA expression in rat EPCs (control) and rat EPCs transfected with miR-21 or miR-control (n = 3). E. WWP1 protein expression in rat EPCs (control) and rat EPCs transfected with miR-control, miR-21, siNC, or siWWP1 by western blotting. $^{**}P < 0.01$ vs. miR-control.

CD133, and the endothelial lineage marker VEGF receptor-2 (VEGFR-2, KDR) by flow cytometric analysis.

Cell proliferation under hypoxic and normoxic conditions

Rat EPCs were seeded in 96-well plates and transfected with 20 nM scramble microRNA (miR-control) or 20 nM anti-miR-21 (Ambion, USA) using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. After the transfection, cells were cultured in supplemented M199 medium at 37°C under normoxic (20% O_2) or hypoxic (1% O_2) conditions for up to 7 days. To determine cell viability, cells were incubated for 4 h with 5 mg/ml MTT (Sigma) solution. The MTT solution was removed, and 150 μl of sterile DMSO (Sigma) was added to each well to dissolve the formazan crystals by incubation at 37°C for 15 min. Absorbance at 570 nm was recorded on an Easy Reader 340 AT (SLT-Lab Instruments), and a growth curve for the cells was established. Experiments were performed in triplicate.

RNA extraction and quantitative real-time PCR

Total RNA was extracted from EPCs using Trizol reagent (Invitrogen) according to the manufacturer's instructions. RNA was reversely transcribed to cDNA using stem-loop primers and the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA). MiR-21 and WWP1 expressions were determined by quantitative real-time PCR using the SYBR Green Master Mix kit (Roche Diagnostics, USA) on an ABI PRISM 7300 system (Applied Biosystems). U6 and GAPDH were used as internal controls for miR-21 and WWP1 expression, respectively. Primers for hsa-miR-21 and U6 were purchased from Applied Biosystems. Primers for WWP1 and GAPDH were designed as follows: rat WWP1: sense, 5'-ACGATACCTCTATTCGGCTT-3';

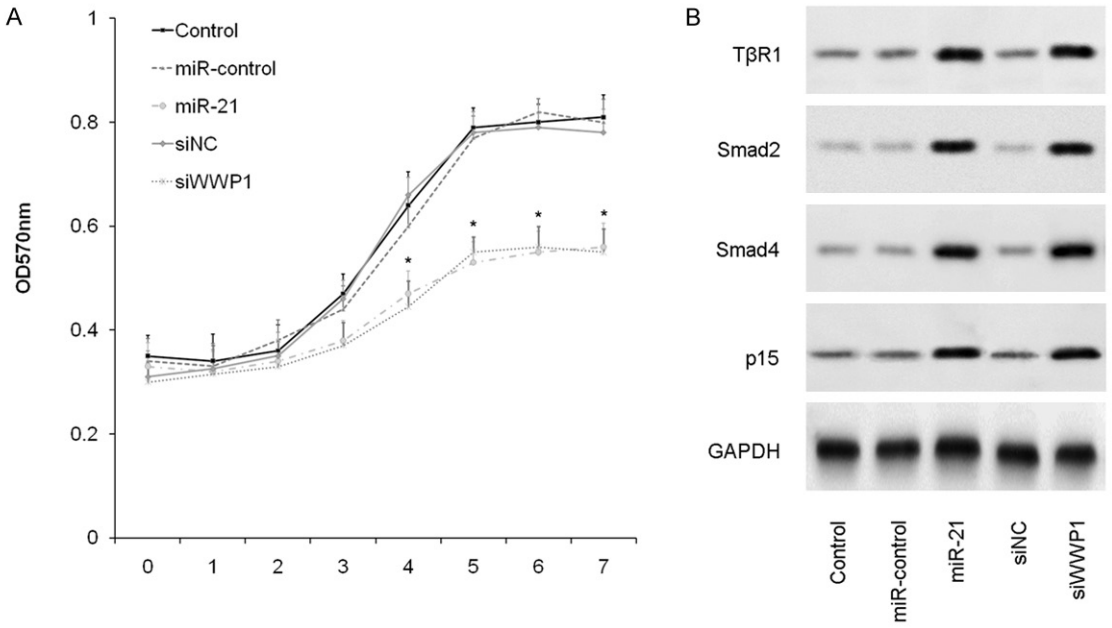

Figure 4. MiR-21 overexpression or WWP1 knockdown inhibits EPC proliferation and activates TGFβ signaling. Rat EPCs were transfected with miR-21 mimic (miR-21), scramble microRNA (miR-control), WWP1 siRNA (siWWP1) or scramble siRNA (siNC). A. Cell proliferation by MTT assay (n = 3). *$P < 0.05$ vs. miR-control. B. Levels of key proteins involved in TGFβ signaling by western blotting.

anti-sense, 5'-CGGGACACATTGATCTTTAC-3'. Rat GAPDH: sense, 5'-CCATCACTGCCACTCAG-AAGA-3'; antisense, 5'-ATACATTGGGGGTAGG-AACAC-3'.

Luciferase reporter assay

The full-length 3'-UTR of rat WWP1 gene containing the putative miR-21 binding site (WWP1-WT-UTR) was amplified from rat genomic DNA by PCR. The 3'-UTR of rat WWP1 containing a mutant miR-21 binding side (WWP1-MUT-UTR) was created using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, USA). WWP1-WT-UTR and WWP1-MUT-UTR were subcloned into the pGL3-Basic plasmid (Promega, America) at the XbaI site (WWP1-WT and WWP1-MUT, respectively). Rat EPCs were seeded in 96-well clusters and co-transfected with 10 nM miR-21 or miR-control mimics (Ambion) and 10 ng of WWP1-WT or WWP1-MUT using Lipofectamine 2000. Luciferase activity was detected 48 h after transfection using a dual luciferase reporter assay system (Promega) and normalized to Renilla activity.

MiR-21 overexpression and WWP1 silencing

To study the effects of miR-21 and WWP1 on TGFβ signaling and cell proliferation of EPCs, rat EPCs were plated into 6-well plates (2.5 × 10^5 cells/well), incubated overnight, and transfected with 10 nM miR-21 mimic (miR-21) or WWP1 siRNA (siWWP1) using Lipofectamine 2000 (Invitrogen). A scramble microRNA (miR-control) and a scramble siRNA (siNC) were used as negative controls. MiR-21 and miR-control were purchased from Ambion, and siWWP1 and siNC were from OriGene Technologies, Inc., USA.

Western blotting

Cells were washed, collected, and lysed on ice for 30 min in modified radioimmune precipitation assay buffer (Applygen Technologies Inc., Beijing, China) containing a protease inhibitor cocktail (Fermentas, USA). After brief sonication, cell lysates were centrifuged at 15,000 g, 4°C for 10 min. The supernatant was collected, and the protein concentration was determined using the BCA Protein Assay kit (Pierce, USA). Samples (50-80 μg total protein) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10-15%) and transferred to nitrocellulose membranes (Millipore, USA). After blocking with 5% nonfat dry milk in TBST (10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 1 h, the membranes

were probed with antibodies against WWP1, TGFβ receptor 1 (TβR1), Smad4, Smad2, p15, and GAPDH, respectively, by overnight incubation at 4°C. After washing with TBST, the membranes were incubated with HRP-conjugated secondary antibodies (Zhongshan Biotechnologies Inc., China) at room temperature for 1 h. Protein bands were visualized using chemiluminescent substrate (Millipore, USA). The antibody against WWP1 was purchased from Abnova, USA. The antibodies against TβR1 and Smad4 were from Cell Signaling, USA. The antibodies against Smad2, p15, and GAPDH were from Santa Cruz Biotechnology, USA.

Statistical analysis

All data are presented as mean + standard derivation (SD). The Student's t-test was used to compare results from different groups. Differences with a P value less than 0.05 were considered statistically significant.

Results

MiR-21 expression is upregulated in EPCs of atherosclerosis patients and EPCs exposed to hypoxia

Circulating EPCs were isolated from peripheral blood of atherosclerosis patients and healthy controls using a Becton-Dickinson FACS Aria cytometer. EPCs of patients and control subjects displayed similar cell morphology under a microscope (**Figure 1A**). In addition, both EPCs expressed stem cell markers CD34 and CD133, and the endothelial lineage marker KDR (**Figure 1B**). However, compared with EPCs of healthy controls, EPCs of atherosclerosis patients showed significantly higher expression of miR-21 ($P < 0.01$, **Figure 1D**) as well as decreased growth rate in culture ($P < 0.05$, **Figure 1C**), suggesting that miR-21 may have a regulatory role in the ability of EPC to proliferate. Hypoxia is an important microenvironmental factor influencing atherosclerosis progression. We subsequently studied the effects of hypoxia treatment on EPCs isolated from rat BMMCs. We found that exposure to hypoxia significantly increased miR-21 expression and decreased cell proliferation of rat EPCs (**Figure 2A**, **2B**). Sequestration of miR-21 in rat EPCs reversed hypoxia-induced growth inhibition (**Figure 2B**). Taken together, these data suggest that increased miR-21 expression suppresses EPC proliferation under hypoxic conditions.

MiR-21 directly regulates WWP1 expression by targeting the 3'-UTR of WWP1

WWP1, a negative regulator of TGFβ signaling, is a putative target of miR-21 [19]. WWP1 is downregulated in human pulmonary artery smooth muscle cells overexpressing miR-21, and is implicated in the regulation of hypoxia-induced pulmonary vascular remodeling by miR-21 [20]. To test whether WWP1 is a direct target of miR-21 in EPCs, we prepared a luciferase reporter expression system using the 3'-UTR of rat WWP1 containing the putative miR-21 binding site (WWP1-WT-UTR) (**Figure 3A**). We also created a luciferase expression system using 3'-UTR of rat WWP1 containing a mutant miR-21 binding side (WWP1-MUT-UTR) (**Figure 3A**). Co-transfection of the luciferase reporter system containing WWP1-WT-UTR (WWP1-WT) with miR-21 in rat EPCs resulted in approximately 50% loss of luciferase reporter expression compared with miR-control ($P < 0.01$, **Figure 3B**). However, luciferase expression was not affected by co-transfection with miR-21 when WWP1-WT-UTR was replaced with WWP1-MUT-UTR in the luciferase reporter system (**Figure 3B**). Further, miR-21 overexpression in rat EPCs significantly suppressed mRNA and protein expression of WWP1, similar to the results obtained with siWWP1 transfection (**Figure 3C-E**). Taken together, these results indicate that miR-21 negatively regulates WWP1 expression in EPCs by directly targeting the 3'-UTR of the WWP1 gene.

MiR-21 inhibits EPC proliferation by activating the TGFβ signaling pathway

Having shown that sequestration of miR-21 reversed hypoxia-induced growth inhibition in rat EPCs, we investigated the effects of miR-21 overexpression on EPC growth under normoxic conditions. We found that rat EPCs transfected with miR-21 proliferated at a significantly slower rate than EPCs transfected with miR-control ($P < 0.05$, **Figure 4A**). Meanwhile, siWWP1 transfection, which resulted in similar loss in WWP1 expression, caused similar decrease in EPC growth rate (**Figure 4A**). These data suggest that the inhibitory effects of miR-21 on EPC proliferation are mediated by WWP1 downregulation. WWP1 is a negative regulator of TGFβ signaling. WWP1 works with Smad7 to cause degradation of TβR1 and inhibits TGFβ-induced phosphorylation of Smad2 [12]. TGFβ can induce antiproliferative gene responses in

many cell types including endothelial cells [21]. Therefore, we speculated that the inhibitory effects of miR-21 on EPC growth are mediated by activation of the TGFβ signaling pathway following WWP1 downregulation. To test this hypothesis, we determined the level of key proteins involved in TGFβ signaling by western blotting. Our results showed that miR-21 or siWWP1 transfection of EPCs led to increased protein levels of TβR1, Smad2, Smad4, and the CDK inhibitor p15, a downstream target molecule of TGFβ (**Figure 4B**). Taken together, our findings indicate that the inhibitory effects of miR-21 on EPC proliferation are mediated by WWP1 downregulation and subsequent activation of the TGFβ signaling pathway.

Discussion

In this study, we found that EPCs of atherosclerosis patients and EPCs exposed to hypoxia have increased expression of miR-21 as well as diminished ability to proliferate. MiR-21 knockdown rescued hypoxia-induced growth arrest in EPCs. These findings indicate that miR-21 likely plays a significant role in diminished vitality of EPCs under pathologic cardiovascular conditions such as atherosclerosis. Next, we examined the regulatory relationship between miR-21 and WWP1, a negative regulator of TGFβ signaling, using a luciferase reporter assay. Co-transfection of miR-21 with a luciferase reporter system containing the 3'-UTR of WWP1 resulted in suppression of luciferase expression; however, the luciferase activity was not affected when the wild type 3'-UTR was replaced with a 3'-UTR containing mutations at the putative miR-21 binding site. Further, the mRNA and protein expression of WWP1 was downregulated in EPCs overexpressing miR-21. These data indicate that WWP1 is a direct target of miR-21 in EPCs. Finally, miR-21 overexpression or WWP1 knockdown in EPCs significantly activates the TGFβ signaling pathway and inhibits cell proliferation. Taken together, our results suggest that the inhibitory effects of miR-21 on EPC proliferation are mediated by downregulation of WWP1 and subsequent activation of the TGFβ signaling pathway.

A number of studies have reported the regulation of EPC function by miRNAs. For example, several angiogenesis-related microRNAs are dyregulated in EPCs of patients with coronary artery diseases [22]. MiR-107 expression is increased in EPCs exposed to hypoxia, and upregulation of miR-107 antagonizes hypoxia-induced EPC differentiation by targeting hypoxia-inducible factor-1 [23]. MiR-221 expression is significantly upregulated in EPCs of patients with coronary artery disease, and overexpression of miR-221 decreases EPC proliferation by downregulating 21/Cdc42/Rac1-activated kinase 1 (PAK1) [18]. Specifically, miR-21 and miR-10A* have been reported to modulate EPC senescence via suppressing high-mobility group A2 [17]. Our findings suggest that upregulation of miR-21 possibly contributes to the reduced availability of EPCs in people with cardiovascular diseases. In addition, our results indicate that miR-21, TGFβ, and their interplay have a significant role in the regulation of EPC proliferation. The effects of miR-21 and TGFβ on other functions of EPC such as adhesion and differentiation remain to be investigated.

Studies have shown that the number of EPCs can be increased by priming with specific growth factors and genetic modifications, as well as life style modifications and pharmaceutical therapies [24, 25]. In this study, we identified a new mechanism regulating the proliferation of EPCs, which may be exploited to enhance the vitality of EPCs for therapeutic applications.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81270003, 81470390) and the Program of Science and Technology Commission of Shanghai Municipality 13ZR1414500 and 11ZR1433200.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bin He, Department of Anesthesiology and SICU, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Kongjiang Road 1665, Shanghai 200092, China. Tel: +86-21-25077825; E-mail: hebinicu@139.com; Dr. Kangkang Zhi, Department of Vascular and Endovascular Surgery, Changzheng Hospital, Shanghai 200003, China. Tel: +86-13816263943; E-mail: kangkang_zhi@163.com

References

[1] Butt M, Dwivedi G, Blann A, Khair O and Lip GY. Endothelial dysfunction: methods of assess-

ment & implications for cardiovascular diseases. Curr Pharm Des 2010; 16: 3442-3454.
[2] Pompilio G, Capogrossi MC, Pesce M, Alamanni F, DiCampli C, Achilli F, Germani A and Biglioli P. Endothelial progenitor cells and cardiovascular homeostasis: clinical implications. Int J Cardiol 2009; 131: 156-167.
[3] Bakogiannis C, Tousoulis D, Androulakis E, Briasoulis A, Papageorgiou N, Vogiatzi G, Kampoli AM, Charakida M, Siasos G, Latsios G, Antoniades C and Stefanadis C. Circulating endothelial progenitor cells as biomarkers for prediction of cardiovascular outcomes. Curr Med Chem 2012; 19: 2597-2604.
[4] Dzau VJ, Gnecchi M, Pachori AS, Morello F and Melo LG. Therapeutic potential of endothelial progenitor cells in cardiovascular diseases. Hypertension 2005; 46: 7-18.
[5] Xu JY, Lee YK, Wang Y and Tse HF. Therapeutic application of endothelial progenitor cells for treatment of cardiovascular diseases. Curr Stem Cell Res Ther 2014; 9: 401-414.
[6] Hayashi H, Abdollah S, Qiu Y, Cai J, Xu YY, Grinnell BW, Richardson MA, Topper JN, Gimbrone MA Jr, Wrana JL and Falb D. The MAD-related protein Smad7 associates with the TGFbeta receptor and functions as an antagonist of TGFbeta signaling. Cell 1997; 89: 1165-1173.
[7] Nikol S, Isner JM, Pickering JG, Kearney M, Leclerc G and Weir L. Expression of transforming growth factor-beta 1 is increased in human vascular restenosis lesions. J Clin Invest 1992; 90: 1582-1592.
[8] Bobik A, Agrotis A, Kanellakis P, Dilley R, Krushinsky A, Smirnov V, Tararak E, Condron M and Kostolias G. Distinct patterns of transforming growth factor-beta isoform and receptor expression in human atherosclerotic lesions. Colocalization implicates TGF-beta in fibrofatty lesion development. Circulation 1999; 99: 2883-2891.
[9] Dabek J, Kulach A, Monastyrska-Cup B and Gasior Z. Transforming growth factor beta and cardiovascular diseases: the other facet of the 'protective cytokine'. Pharmacol Rep 2006; 58: 799-805.
[10] Sales VL, Engelmayr GC, Mettler BA, Johnson JA, Sacks MS and Mayer JE Jr Transforming growth factor-beta1 modulates extracellular matrix production, proliferation, and apoptosis of endothelial progenitor cells in tissue-engineering scaffolds. Circulation 2006; 114: 193-199.
[11] Redondo S, Hristov M, Gumbel D, Tejerina T and Weber C. Biphasic effect of pioglitazone on isolated human endothelial progenitor cells: involvement of peroxisome proliferator-activated receptor-gamma and transforming growth factor-beta1. Thromb Haemost 2007; 97: 979-987.
[12] Komuro A, Imamura T, Saitoh M, Yoshida Y, Yamori T, Miyazono K and Miyazawa K. Negative regulation of transforming growth factor-beta (TGF-beta) signaling by WW domain-containing protein 1 (WWP1). Oncogene 2004; 23: 6914-6923.
[13] Cao X, Xue L, Han L, Ma L, Chen T and Tong T. WW domain-containing E3 ubiquitin protein ligase 1 (WWP1) delays cellular senescence by promoting p27 (Kip1) degradation in human diploid fibroblasts. J Biol Chem 2011; 286: 33447-33456.
[14] Liu LZ, Li C, Chen Q, Jing Y, Carpenter R, Jiang Y, Kung HF, Lai L and Jiang BH. MiR-21 induced angiogenesis through AKT and ERK activation and HIF-1alpha expression. PLoS One 2011; 6: e19139.
[15] Zhao D, Tu Y, Wan L, Bu L, Huang T, Sun X, Wang K and Shen B. In vivo monitoring of angiogenesis inhibition via down-regulation of mir-21 in a VEGFR2-luc murine breast cancer model using bioluminescent imaging. PLoS One 2013; 8: e71472.
[16] Sabatel C, Malvaux L, Bovy N, Deroanne C, Lambert V, Gonzalez ML, Colige A, Rakic JM, Noel A, Martial JA and Struman I. MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. PLoS One 2011; 6: e16979.
[17] Zhu S, Deng S, Ma Q, Zhang T, Jia C, Zhuo D, Yang F, Wei J, Wang L, Dykxhoorn DM, Hare JM, Goldschmidt-Clermont PJ and Dong C. MicroRNA-10A* and MicroRNA-21 modulate endothelial progenitor cell senescence via suppressing high-mobility group A2. Circ Res 2013; 112: 152-164.
[18] Zhang X, Mao H, Chen JY, Wen S, Li D, Ye M and Lv Z. Increased expression of microRNA-221 inhibits PAK1 in endothelial progenitor cells and impairs its function via c-Raf/MEK/ERK pathway. Biochem Biophys Res Commun 2013; 431: 404-408.
[19] Ma X, Kumar M, Choudhury SN, Becker Buscaglia LE, Barker JR, Kanakamedala K, Liu MF and Li Y. Loss of the miR-21 allele elevates the expression of its target genes and reduces tumorigenesis. Proc Natl Acad Sci U S A 2011; 108: 10144-10149.
[20] Yang S, Banerjee S, Freitas A, Cui H, Xie N, Abraham E and Liu G. miR-21 regulates chronic hypoxia-induced pulmonary vascular remodeling. Am J Physiol Lung Cell Mol Physiol 2012; 302: L521-529.
[21] Massague J, Blain SW and Lo RS. TGFbeta signaling in growth control, cancer, and heritable disorders. Cell 2000; 103: 295-309.
[22] Zhang Q, Kandic I and Kutryk MJ. Dysregulation of angiogenesis-related microRNAs in endo-

thelial progenitor cells from patients with coronary artery disease. Biochem Biophys Res Commun 2011; 405: 42-46.

[23] Meng S, Cao J, Wang L, Zhou Q, Li Y, Shen C, Zhang X and Wang C. MicroRNA 107 partly inhibits endothelial progenitor cells differentiation via HIF-1beta. PLoS One 2012; 7: e40323.

[24] Hung HS, Shyu WC, Tsai CH, Hsu SH and Lin SZ. Transplantation of endothelial progenitor cells as therapeutics for cardiovascular diseases. Cell Transplant 2009; 18: 1003-1012.

[25] Umemura T and Higashi Y. Endothelial progenitor cells: therapeutic target for cardiovascular diseases. J Pharmacol Sci 2008; 108: 1-6.