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MiR-92a regulates viability and angiogenesis of endothelial cells under oxidative stress

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ABSTRACT

Oxidative stress contributes to endothelial cell (EC) dysfunction, which is prevalent in ageing and atherosclerosis. MicroRNAs (miRs) are small, non-coding RNAs that post-transcriptionally regulate gene expression and play a key role in fine-tuning EC functional responses, including apoptosis and angiogenesis. MiR-92a is highly expressed in young endothelial cells in comparison with senescent endothelial cells, which exhibit increased oxidative stress and apoptosis. However, the impact of miR-92a treatment on EC viability and angiogenesis under oxidative stress is unknown.

Hydrogen peroxide (H_2O_2) was used to induce oxidative stress in human umbilical vein endothelial cells (HUVEC). Pre-miR-92a treatment decreased H_2O_2-induced apoptosis of HUVEC as determined by TUNEL assay. Pre-miR-92a treatment enhanced capillary tube formation by HUVEC under oxidative stress, which was blocked by LY294002, an inhibitor of Akt phosphorylation. Interestingly, we also observed that inhibition of miR-92a by anti-miR-92a antisense can also enhance angiogenesis in HUVEC with and without oxidative stress exposure. Our results show that perturbation of miR-92a levels outside of its narrow "homeostatic" range may trigger endothelial cell angiogenesis, suggesting that the role of miR-92a in regulating angiogenesis is controversial and may vary depending on the experimental model and method of regulating miR-92a.

1. Introduction

Endothelial cells (ECs) play an important role in vascular homeostasis and modulation of vascular disease, regulating key physiological functions including vasorelaxation, inflammation and angiogenesis. Endothelial dysfunction is an important early event in atherosclerosis and is triggered by diabetes, hypertension, hyperlipidemia and smoking, common risk factors for coronary and peripheral arterial disease [1]. Reactive oxygen species and oxidative stress play a key role in the pathogenesis of endothelial dysfunction [2,3]. Vascular ageing is also characterized by enhanced oxidative stress resulting from alterations in the balance between production and destruction of reactive oxygen species (ROS) [4]. Identifying the mechanisms that control EC viability and function in the setting of oxidative stress and aging are important for developing effective therapeutic strategies against vascular disease.

MicroRNAs (miRNAs) are a recently discovered class of approximately 22-nucleotide regulatory RNAs that post-transcriptionally regulate gene expression [5]. Mature miRNAs can form miRNA-induced silencing complexes, which bind to the 3′-untranslated region (3′UTR) of target mRNAs to mediate translational repression [6]. Previous studies have suggested that miRNAs play important roles in regulating cell apoptosis [7–9] and the progression of vascular disease [10,11]. Amongst the various miRNAs, miR-92a is a component of the miR-17-92 cluster, which is highly expressed in human ECs, particularly in young endothelial cells [12]. MiR-92a was identified as negative regulator of angiogenesis by targeting the α5 integrin subunit (ITGA5) [13]. Murata et al. [14] reported that inhibition of miR-92a enhanced fracture healing by promoting angiogenesis in mice. Conversely, systemic administration of an antagomir to miR-92, which reduced miR-92a expression in skeletal muscle tissues of mice, failed to improve angiogenic responses during mechanical loading [15]. Therefore, the role of miR-92a in regulating EC function and angiogenesis is incompletely defined.

The expression of miR-92a is regulated negatively by oxidative stress, and a recent report demonstrated that radiation-induced

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oxidative stress represses miR-92a expression [16]. MiR-92a expression levels also decline progressively with age. Rippe et al. [12] reported that senescence of human endothelial cells is associated with reduced expression of miR-92a and enhanced apoptosis. Ohyashiki et al. [17] also reported age-related decreases in miR-92a expression in human lymphocytes. Moreover, anti-miR-92a-treated cells exhibited increased apoptosis [18]. Together, these findings suggest that repression of miR-92a during oxidative stress and aging can adversely affect cell viability.

The level of miR-92a expression is inversely correlated with expression of phosphatase and tensin homolog (PTEN) in many tumor tissues [19–21]. The loss of PTEN leads to activation of Akt, also known as Protein kinase B (PKB), a regulator of endothelial cell apoptosis and angiogenesis [22]. In this study, we evaluated the role of miR-92a in regulating endothelial cell viability and angiogenesis under oxidative stress, focusing on expression of PTEN and related mechanisms.

2. Methods

2.1. Cell culture and reagents

HUVEC were obtained from the American Type Culture Collection and cultured in endothelial cell growth media (EGM-2, Lonza) at 37 °C in a humidified atmosphere of 5% CO_2.

2.2. Synthetic miRNA transfection

Pre-miR microRNA are double-stranded RNA mimics, which can be introduced into target cells using electroporation to mimic endogenous mature microRNA molecular in cells. For miRNA overexpression experiments, 100 nM pre-miR-92a mimic (GenePharma, China) or pre-miR-scramble control (NC), was transfected into HUVEC in 100 μL "R buffer" using Neon electroporation transfection system (Invitrogen) operating at 1350 V with one 30 ms pulse. For miRNA anti-sense inhibitor experiments, 100nM single-stranded 2′ *O*-methyl enhanced miR-92a or control inhibitor (GenePharma, China) was transfected into HUVEC using Neon transfection system.

2.3. Immunofluorescence and confocal microscopy

For cell staining, cells were plated on 8-well chamber slides (Millipore, Billerica, MA) and subjected to oxidative stress treatments as described above. (1) The terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick-end-labeling (TUNEL) staining for apoptotic nuclei was performed using DEAD End TUNEL kit (Promega, Madison, WI) according to the manufacturer's instruction with modification. Briefly, cells were fixed in 4% paraformaldehyde for 25 min and then treated with permeabilization solution (0.2% Triton X-100 solution in PBS) for 5 min at room temperature. Labeling reactions were performed with 100 μl of reaction buffer for 60 min at 37 °C in a humidified chamber, followed by steptavidin Alexa Fluor 555 conjugate (1:400, Life Technologies, Carlsbad, CA) staining. Slides were mounted using VECTASHIELD HardSet Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA). The staining was analyzed by Zeiss 510 Laser Scanning Microscope (Carl Zeiss, Thornwood, NY). Apoptosis was evaluated as the average number of positively stained cells per DAPI labeled cells.

2.4. Production of lentiviral GFP vector and HUVEC infection

To visualize tube formation for in vitro angiogenesis assays, HUVEC were genetically engineered to express GFP. Viral particles were produced by transfection of 293FT cells (Invitrogen) with pRRLSIN.cPPT.PGK-GFP.WPRE (Addgene 12252), an expression plasmid, together with an envelope plasmid (pMD2.G, Addgene 12259) and a packaging plasmid (psPAX2, Addgene 12260) with Fugene HD (Roche). Virus-containing medium was collected 48 h after transfection on 2 consecutive days, passed through a 0.45 μm filter to remove cell debris, and concentrated by ultracentriguation. HUVEC were infected with GFP-expressing lentiviral vector at 8 μg/ml polybrene (Sigma). The GFP-positive HUVEC were purified by FACS sorting.

2.5. Tube formation assay under oxidative stress

Growth factor-reduced (GFR) Matrigel (BD Bioscience) with 0 μM or 600 μM H_2O_2 was coated on 15-well μ-angiogenesis slides at 10 μl/well (ibidi, Germany) to mimic the effects of vascular oxidative stress. The coated slides were incubated for 15 min at 37 °C, seeded with HUVEC (10,000 cells/well), and incubated for 10 h at 37 °C to allow tube formation. LY294002 (50 μM), a PI3K/Akt inhibitor, was used to treat the cells for one hour prior to tube formation assay. The wells were then imaged for capillary-like structures using an EVOS microscope (Life Technologies). Quantification of the tubes was performed by taking 4× images of each chamber followed by image analysis using Image J.

2.6. Protein extraction and western blot analysis

Cells were lysed in RIPA buffer (50 mM Tris–HCl, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, PH 8.0) supplemented with a protease inhibitor cocktail (Roche Applied Science). The protein concentration was measured using a Bradford protein assay kit (Coomassie Plus Protein Assay reagent, Thermo). Protein samples were separated by 10% SDS–PAGE (Bio-Rad) and electroblotted onto 0.45 μm Immobilon polyvinylidene difluoride (PVDF) membranes (Millipore). The membranes were blocked with 5% Blotting-Grade Blocker (Bio-Rad) in PBST for 1 h at room temperature. The membrane was incubated with the respective antibodies: rabbit anti-PTEN XP (1:1000; Cell signaling,), rabbit anti-phospho-Akt (S473, 1:2,000; cell signaling), rabbit anti-Akt1 (1:500; Thermo) and mouse anti-GAPDH antibody (1:4,000; Millipore) overnight at 4 °C. Membranes were then incubated for 1 h at room temperature with Amersham ECL peroxidase-lined secondary antibodies: sheep anti-mouse IgG (1:10,000, GE Healthcare) or donkey anti-rabbit IgG (1:10,000, GE Healthcare). Western blot immunoreactivity was detected using a Super Signal West Femto Maximum Sensitivity Substrate Kit (Thermo) in C-DiGit Blot Scanner (LI-COR Biosciences). The density of protein bands was measured using Image J software, and values were normalized to the densitometric values of GAPDH or total Akt1 in samples.

2.7. Statistical analysis

Results are presented as the mean ± SEM. Comparisons between groups were made by one-way analysis of variance or two-tailed Student's *t* test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Protective effects of pre-miR-92a treatment in endothelial cells against H_2O_2-induced apoptosis

To investigate the effects of miR-92a on endothelial cell viability under oxidative stress, HUVEC were transfected with pre-miR-92a or pre-miR-NC for two days, and then exposed to 200 μM H_2O_2 for 16hrs. We found that pre-miR-92a treatment

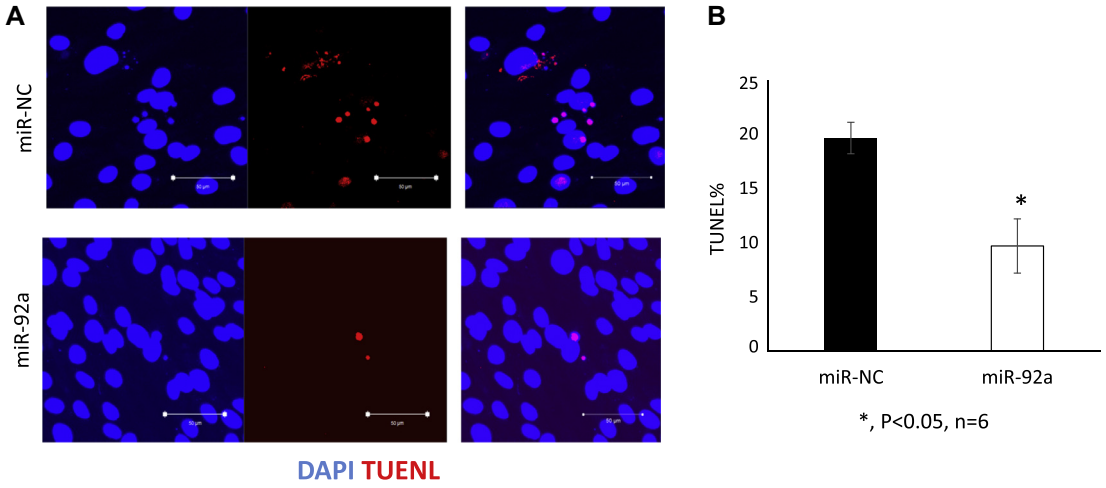

Fig. 1. Effects of pre-miR-92a treatment on H_2O_2-induced apoptosis (200 μM H_2O_2 for 16 h) in HUVEC. (A) HUVEC were stained with TUNEL reagent (red) and counterstained with DAPI (blue); (B) Comparison of percentage of TUNEL-positive HUVEC pretreated with pre-miR-92a versus pre-miR-NC. Values are expressed as mean ± SEM, $n = 6$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significantly decreased apoptotic cells assessed by TUNEL staining (Fig. 1A and B).

3.2. Overexpression of MiR-92a has insignificant effect on angiogenesis of HUVEC

To investigate the effect of miR-92a on HUVEC-mediated angiogenesis, in vitro Matrigel tube formation assay was performed. Stable GFP labeling of HUVEC was performed with a lentiviral vector system to facilitate quantification of angiogenesis. After infection with the lentiviral vectors, the GFP-positive HUVEC were purified by FACS sorting. The expression of the exogenous gene GFP was confirmed under fluorescence microscopy. As illustrated in Fig. 2A, almost all HUVEC express GFP. As illustrated in Fig. 2B, treatment with pre-miR-92a, in comparison with pre-miR-NC, had a small but insignificant effect on capillary tube formation under normal culture conditions, in the absence of H_2O_2. We also investigated the effect of miR-92a inhibition on HUVEC-mediated angiogenesis, consistent with previous study by Bonauer et al. [13], miR-92a inhibition by anti-miR-92a can significantly increase the capillary formation under normal culture conditions (Fig. 2C).

3.3. Preservation of endothelial cell angiogenesis in the setting of oxidative stress by miR-92a: role of Akt

Given the ability of miR-92a to protect HUVEC from H_2O_2-induced apoptosis, we next examined whether miR-92a treatment can preserve HUVEC-induced angiogenesis in the setting of oxidative stress. We performed in vitro angiogenesis assays using matrigel containing impregnated with H_2O_2 to mimic the effects of vascular oxidative stress (Fig. 3A). As illustrated in Fig. 3B and C, exposure to H_2O_2 profoundly impaired tube-forming ability by pre-miR-NC treated HUVEC, which was significantly reduced by treatment with pre-miR-92a (Fig. 3F and G). Inhibition of Akt phosphorylation by Ly294002 abrogated the protective effects of pre-miR-92a on angiogenesis in the setting of oxidative stress (Fig. 3D–G), suggesting a key mechanistic role for Akt signaling. Moreover, we examined whether miR-92a inhibition can preserve HUVEC-induced angiogenesis in the setting of oxidative stress. As illustrated in Fig. 3H and I, exposure to H_2O_2 profoundly impaired tube-forming ability by anti-miR-NC treated HUVEC, which was significantly reduced by treatment with anti-miR-92a (Fig. 3L and M), in addition, inhibition of Akt phosphorylation by Ly294002 abrogate most of the protective effects of anti-miR-92a on angiogenesis in the setting of oxidative stress, suggesting that some protective effects of anti-miR-92a on angiogenesis are also dependent on Akt signaling (Fig. 3J–M).

3.4. MiR-92a inhibits PTEN expression and promotes Akt1 phosphorylation

PTEN negatively regulates cell survival and angiogenesis by inhibition of the Akt signaling pathway [23,24]. An inverse correlation between the expression levels of PTEN and miR-92 has been reported in many human cancer tissues [19–21]. To investigate whether PTEN is a target of miR-92a, we analyzed PTEN protein levels in mi-RNA transfected HUVEC cells by western blot. Compared with pre-miR-NC control, HUVEC treated with pre-miR-92a showed decreased PTEN expression, and HUVEC treated with anti-miR-92a showed increased PTEN expression (Fig. 4A). Moreover, under these conditions, reduced PTEN expression was associated with higher levels of Akt1 phosphorylation in HUVEC treated with pre-miR-92a while increased PTEN expression was associated with slightly lower levels of Akt1 phosphorylation in HUVEC treated with anti-miR-92a (Fig. 4B).

4. Discussion

MiR-92a, a component of the miR-17-92 cluster, is highly expressed in young healthy human endothelial cells in comparison to senescent endothelial cells, which exhibit enhanced oxidative stress and apoptosis [12]. However, the role of miR-92a in regulating EC viability and angiogenesis under oxidative stress is poorly understood. In this study, we found that pre-miR-92a treatment has protective effects against oxidative stress-induced apoptosis in EC while preserving angiogenic capacity. In addition, pre-miR-92 augmented the Akt signaling pathway by suppressing PTEN, which has been reported as a direct target gene of miR-92a [25].

Oxidative stress induces both EC apoptosis [2,3] and downregulation of miR-92a expression [16]. The relationship between miR-92a and cell viability has received little attention, however, and to our knowledge, no prior studies have examined this relationship in EC. A recent study in human glioma cells reported that antisense inhibition of miR-92a induced cell apoptosis via directly targeting the Bim gene, suggesting a role for miR-92a in regulating viability

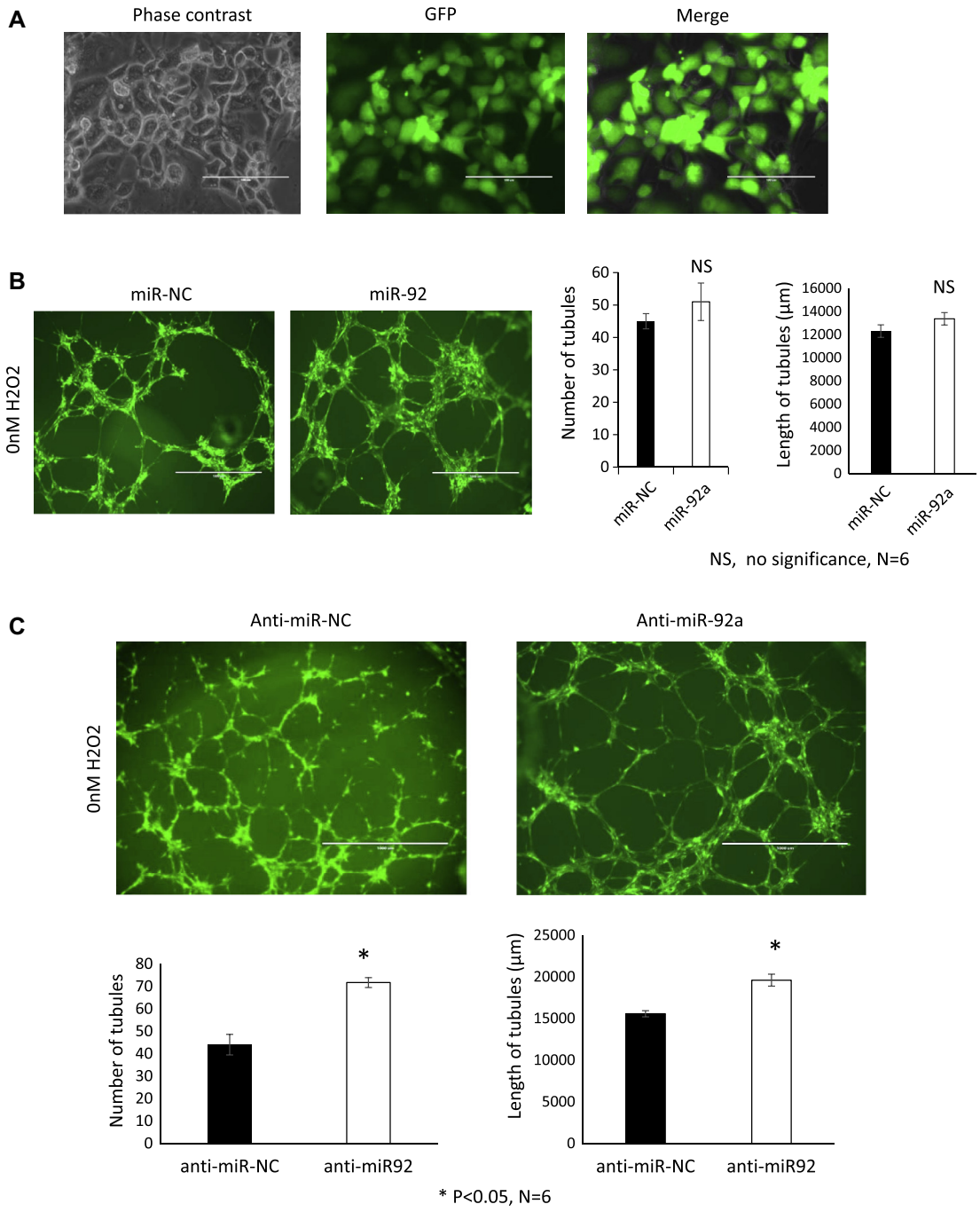

Fig. 2. Effects of miR-92a overexpression and inhibition on capillary tube formation by HUVEC without oxidative stress. (A) GFP transgene expression of HUVEC infected with lentiviral vectors; (B) HUVEC were seeded on Matrigel without H_2O_2 and treated with NC control or pre-miR-92a; (C) HUVEC were seeded on Matrigel without H2O2 and treated with anti-miR-NC control or anti-miR-92a, Values are expressed as mean ± SEM, $n = 6$.

of malignant cells [26]. Our findings with miR-92a overexpression in EC are consistent with these latter findings. The PTEN/Akt pathway was reported to play a key role in cell survival under oxidative or nitrosative stress [27], with oxidative stress-mediated cell apoptosis correlating with increased PTEN expression and inhibition of AKT signaling [28]. PTEN gene deletion was reported to enhance cell survival by activation of the Akt pathway [29]. PTEN is the target of certain cellular miRNAs, including miR-19a/b [30], miR-29a [31], miR-214 [32], miR-205 [33] and miR-494 [34], which can directly suppress the expression of PTEN in a post-transcriptional manner. An inverse correlation between PTEN and miR-92 expression has been observed in many human cancer tissues, which typically express low levels of PTEN protein and high levels of miR-92a [19–21]. In the present study, we found that miR-92a overexpression enhanced endothelial cell viability under oxidative stress via augmenting Akt signaling, suggesting a vasculoprotective effect that is likely mediated through a PTEN-dependent mechanism.

In 2009, Bonauer et al. [13] demonstrated that α5 integrin is a direct target of miR-92a, and that inhibition of miR-92a could enhance blood vessel growth and functional recovery of ischemic tissues. Likewise, Ohyagi-Hara et al. [35] recently demonstrated that miR-92 directly targets integrin α5, with transfection of precursor miR-92a reducing integrin α5 expression and inhibiting cancer cell adhesion, invasion, and proliferation. Conversely, Sengul et al. [15] reported that systemic administration of a miR-92 antagomir reduced mir-92a levels in skeletal muscle tissue

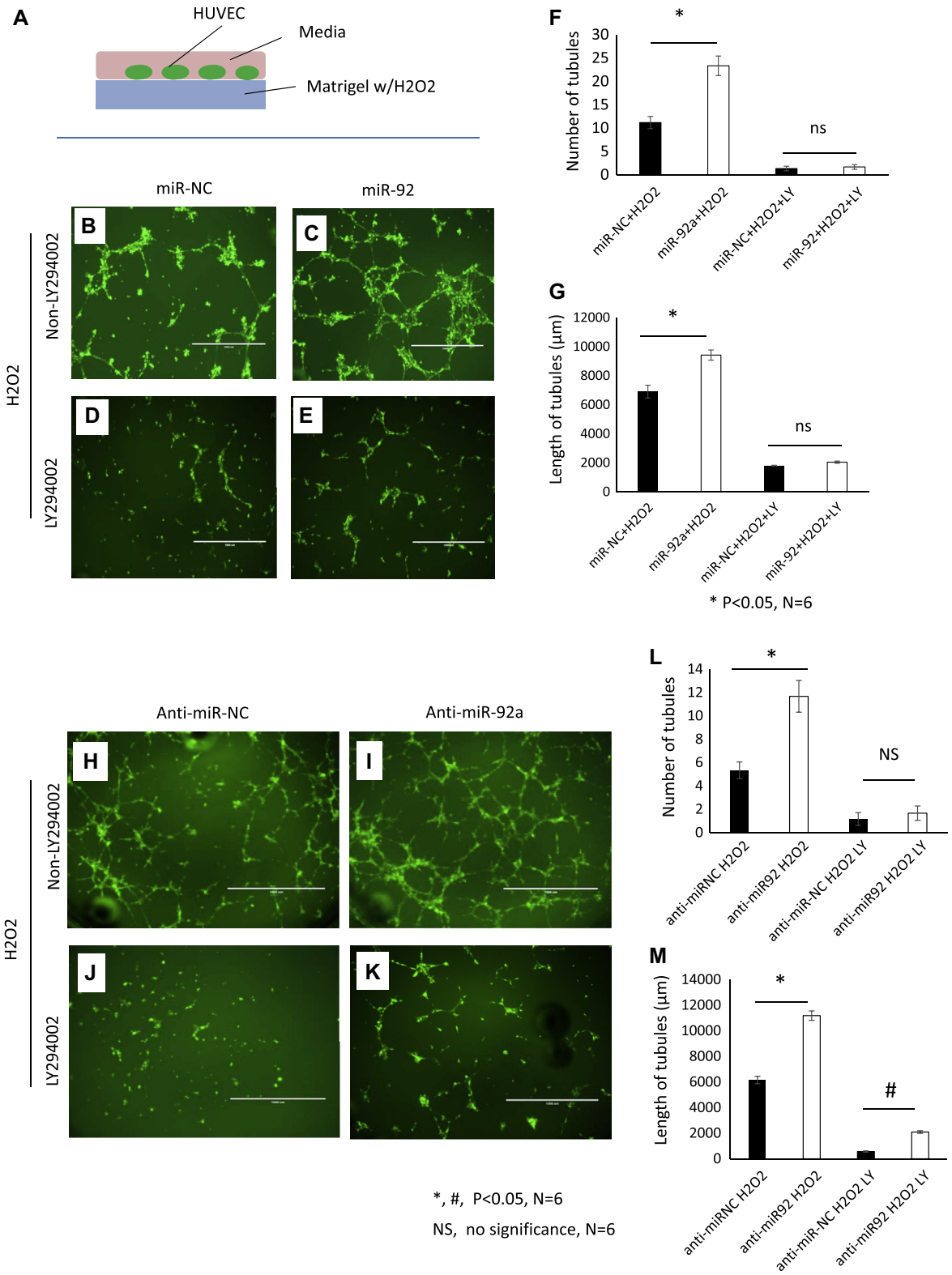

Fig. 3. Effects of pre-miR-92a treatment and anti-miR-92a inhibition on capillary tube formation by HUVEC under oxidative stress. (A) Diagram of in vitro angiogenesis model system; (B–E) NC control or pre-miR-92a treated HUVEC were seeded on Matrigel with H_2O_2 in the absence or presence of LY294002. Representative images are shown in B–E, and data are quantified in F (tubule numbers) and G (tubule length); (H–K) anti-miR-NC control or anti-miR-92a treated HUVEC were seeded on Matrigel with H_2O_2 in the absence or presence of LY294002. Representative images are shown in H–K, and data are quantified in L (tubule numbers) and M (tubule length). Values are expressed as mean ± SEM; $^*P < 0.05$, $n = 6$, NS = no significant difference.

but failed to enhance angiogenic responses in mice. Therefore, the role of miR-92a in regulating angiogenesis is controversial and may vary depending on the experimental model and method of regulating miR-92a [36].

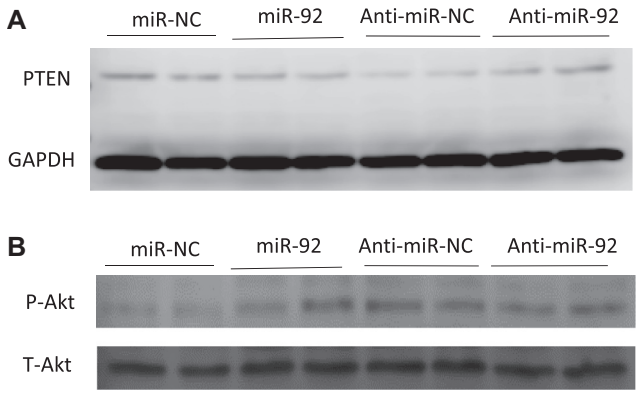

Fig. 4. Western blot analysis of PTEN (normalized to GAPDH) (A) and Phos-Akt1 (normalized to total Akt1) (B) in extracts from HUVEC treated with miR-NC, pre-miR-92a, anti-miR-NC and anti-miR-92a.

The classic PTEN/Akt pathway also plays a central role in regulating angiogenesis [37]. PTEN down-regulates the AKT signaling pathway by dephosphorylating phosphatidylinositol 3, 4, 5-trisphosphate (PIP_3), which leads to reduced phosphorylation and diminished activation of Akt. Elevated PTEN is believed to be a major factor responsible for aging-related impairment of angiogenesis [23]. In the present study, we report that miR-92a overexpression preserves the angiogenic capacity of EC under oxidative stress. Moreover, we demonstrate that miRNA-92a overexpression effectively suppresses PTEN expression in HUVEC. Interestingly, we also observed that inhibition of miR-92a by anti-miR-92a antisense can also enhance angiogenesis in HUVEC exposed to oxidative stress. Thus, perturbation of miR-92a levels outside of its narrow "homeostatic" range may trigger endothelial cell angiogenesis. It is not surprising that both overexpression and downregulation of miR-92a could have pro-angiogenic effects in HUVEC. MiR-92a has hundreds of predicted targets that potentially can positively or negatively regulate angiogenesis, suggesting that alterations in expression of miR-92a in either direction could potentially induce angiogenesis. EC express high levels of miR-92a under basal conditions, and it is possible that pre-miR-92a treatment may not produce major changes in angiogenesis if its targets (i.e., PTEN) are already repressed. In the setting of oxidative stress, however, PTEN is upregulated, and the effects of miR-92a overexpression to repress PTEN expression likely play a dominant role to promote angiogenesis. In this study, we also observed that inhibition of Akt phosphorylation by Ly294002 abrogates most of the protective effects of anti-miR-92a on angiogenesis in the setting of oxidative stress, it is not surprising because major biological function of ITGA5, the verified miR-92a target, is also dependent on PI3K/Akt pathway [38].

In conclusion, our results indicate that the pre-miR-92a treatment improves HUVEC viability and preserves angiogenic capacity under oxidative stress, at least partially through down-regulation of PTEN expression. Our data suggest that modulation of endothelial cell function by miR92a depends on the cellular environment, which likely has major effects on expression of miR92a target genes. Further studies are needed to determine whether miR92a-based therapeutic strategies could be exploited to protect endothelial cell survival and function in the setting of enhanced oxidative stress.

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Conflict of interest

None to disclose.

References

[1] K. Sugamura, J.F. Keaney Jr., Reactive oxygen species in cardiovascular disease, Free Radic. Biol. Med. 51 (2011) 978–992.
[2] P.C. Schulze, R.T. Lee, Oxidative stress and atherosclerosis, Curr. Atheroscler. Rep. 7 (2005) 242–248.
[3] D.H. Endemann, E.L. Schiffrin, Endothelial dysfunction, J. Am. Soc. Nephrol. 15 (2004) 1983–1992.
[4] M. Li, N.K. Fukagawa, Age-related changes in redox signaling and VSMC function, Antioxid. Redox Signal. 12 (2010) 641–655.
[5] M. Tatsuguchi, H.Y. Seok, T.E. Callis, J.M. Thomson, J.F. Chen, M. Newman, et al., Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy, J. Mol. Cell. Cardiol. 42 (2007) 1137–1141.
[6] Y. Tang, Y. Wang, L. Chen, Y. Pan, N. Weintraub, Cross talk between the Notch signaling and noncoding RNA on the fate of stem cells, Prog. Mol. Biol. Transl. Sci. 111 (2012) 175–193.
[7] C.C. Chio, J.W. Lin, H.A. Cheng, W.T. Chiu, Y.H. Wang, J.J. Wang, et al., MicroRNA-210 targets antiapoptotic Bcl-2 expression and mediates hypoxia-induced apoptosis of neuroblastoma cells, Arch. Toxicol. 87 (2013) 459–468.
[8] L. Liu, R. Chen, S. Huang, Y. Wu, G. Li, B. Zhang, et al., MiR-153 sensitized the K562 cells to As2O3-induced apoptosis, Med. Oncol. 29 (2012) 243–247.
[9] L. Qian, L.W. Van Laake, Y. Huang, S. Liu, M.F. Wendland, D. Srivastava, MiR-24 inhibits apoptosis and represses Bim in mouse cardiomyocytes, J. Exp. Med. 208 (2011) 549–560.
[10] Y. Cheng, C. Zhang, MicroRNA-21 in cardiovascular disease, J. Cardiovasc. Transl. Res. 3 (2010) 251–255.
[11] V. Jazbutyte, T. Thum, MicroRNA-21: from cancer to cardiovascular disease, Curr. Drug Targets 11 (2010) 926–935.
[12] C. Rippe, M. Blimline, K.A. Magerko, B.R. Lawson, T.J. LaRocca, A.J. Donato, et al., MicroRNA changes in human arterial endothelial cells with senescence. Relation to apoptosis, eNOS and inflammation, Exp. Gerontol. 47 (2012) 45–51.
[13] A. Bonauer, G. Carmona, M. Iwasaki, M. Mione, M. Koyanagi, A. Fischer, et al., MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice, Science 324 (2009) 1710–1713.
[14] K. Murata, H. Ito, H. Yoshitomi, K. Yamamoto, A. Fukuda, J. Yoshikawa, et al., Inhibition of miR-92a enhances fracture healing via promoting angiogenesis in a model of stabilized fracture in young mice, J. Bone Miner. Res. 29 (2013) 316–326.
[15] A. Sengul, R. Santisuk, W. Xing, C. Kesavan, Systemic administration of an antagomir designed to inhibit miR-92, a regulator of angiogenesis, failed to modulate skeletal anabolic response to mechanical loading, Physiol. Res. 62 (2013) 221–226.
[16] M.A. Chaudhry, R.A. Omaruddin, C.D. Brumbaugh, M.A. Tariq, N. Pourmand, Identification of radiation-induced microRNA transcriptome by next-generation massively parallel sequencing, J. Radiat. Res. 54 (2013) 808–822.
[17] M. Ohyashiki, J.H. Ohyashiki, A. Hirota, C. Kobayashi, K. Ohyashiki, Age-related decrease of miRNA-92a levels in human CD8+ T-cells correlates with a reduction of naive T lymphocytes, Immun. Ageing 8 (2011) 11.
[18] J.H. Ohyashiki, T. Umezu, C. Kobayashi, R.S. Hamamura, M. Tanaka, M. Kuroda, et al., Impact on cell to plasma ratio of miR-92a in patients with acute leukemia: in vivo assessment of cell to plasma ratio of miR-92a, BMC Res. Notes 3 (2010) 347.
[19] B. Zhao, Y. Zhu, K. Cui, J. Gao, F. Yu, L. Chen, et al., Expression and significance of PTEN and miR-92 in hepatocellular carcinoma, Mol. Med. Rep. 7 (2013) 1413–1416.
[20] G. Zhang, H. Zhou, H. Xiao, Z. Liu, H. Tian, T. Zhou, MicroRNA-92a functions as an oncogene in colorectal cancer by targeting PTEN, Dig. Dis. Sci. 59 (2014) 98–107.
[21] L. Tian, Y.X. Fang, J.L. Xue, J.Z. Chen, Four microRNAs promote prostate cell proliferation with regulation of PTEN and its downstream signals in vitro, PLoS One 8 (2013) e75885.
[22] P.R. Somanath, O.V. Razorenova, J. Chen, T.V. Byzova, Akt1 in endothelial cell and angiogenesis, Cell Cycle 5 (2006) 512–518.
[23] A.S. Tarnawski, R. Pai, T. Tanigawa, T. Matysiak-Budnik, A. Ahluwalia, PTEN silencing reverses aging-related impairment of angiogenesis in microvascular endothelial cells, Biochem. Biophys. Res. Commun. 394 (2010) 291–296.
[24] A. Suzuki, K. Hamada, T. Sasaki, T.W. Mak, T. Nakano, Role of PTEN/PI3K pathway in endothelial cells, Biochem. Soc. Trans. 35 (2007) 172–176.
[25] G. Zhang, H. Zhou, H. Xiao, Z. Liu, H. Tian, T. Zhou, MicroRNA-92a functions as an oncogene in colorectal cancer by targeting PTEN, Dig. Dis. Sci. 59 (2014) 98–107.
[26] H. Niu, K. Wang, A. Zhang, S. Yang, Z. Song, W. Wang, et al., MiR-92a is a critical regulator of the apoptosis pathway in glioblastoma with inverse expression of BCL2L11, Oncol. Rep. 28 (2012) 1771–1777.
[27] P. Song, Y. Wu, J. Xu, Z. Xie, Y. Dong, M. Zhang, et al., Reactive nitrogen species induced by hyperglycemia suppresses Akt signaling and triggers apoptosis by upregulating phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10) in an LKB1-dependent manner, Circulation 116 (2007) 1585–1595.
[28] Y. Zhang, X. Zhang, Z.N. Rabbani, I.L. Jackson, Z. Vujaskovic, Oxidative stress mediates radiation lung injury by inducing apoptosis, Int. J. Radiat. Oncol. Biol. Phys. 83 (2012) 740–748.

[29] Y. Zhang, A.C. Granholm, K. Huh, L. Shan, O. Diaz-Ruiz, N. Malik, et al., PTEN deletion enhances survival, neurite outgrowth and function of dopamine neuron grafts to MitoPark mice, Brain 135 (2012) 2736–2749.
[30] Z. Liang, Y. Li, K. Huang, N. Wagar, H. Shim, Regulation of miR-19 to breast cancer chemoresistance through targeting PTEN, Pharm. Res. 28 (2011) 3091–3100.
[31] G. Kong, J. Zhang, S. Zhang, C. Shan, L. Ye, X. Zhang, Upregulated microRNA-29a by hepatitis B virus X protein enhances hepatoma cell migration by targeting PTEN in cell culture model, PLoS One 6 (2011) e19518.
[32] L.M. Li, D.X. Hou, Y.L. Guo, J.W. Yang, Y. Liu, C.Y. Zhang, et al., Role of microRNA-214-targeting phosphatase and tensin homolog in advanced glycation end product-induced apoptosis delay in monocytes, J. Immunol. 186 (2011) 2552–2560.
[33] J. Cai, L. Fang, Y. Huang, R. Li, J. Yuan, Y. Yang, et al., MiR-205 targets PTEN and PHLPP2 to augment AKT signaling and drive malignant phenotypes in non-small cell lung cancer, Cancer Res. 73 (2013) 5402–5415.
[34] L. Liu, Y. Jiang, H. Zhang, A.R. Greenlee, Z. Han, Overexpressed miR-494 down-regulates PTEN gene expression in cells transformed by anti-benzo(a)pyrene-trans-7,8-dihydrodiol-9,10-epoxide, Life Sci. 86 (2010) 192–198.
[35] C. Ohyagi-Hara, K. Sawada, S. Kamiura, Y. Tomita, A. Isobe, K. Hashimoto, et al., MiR-92a inhibits peritoneal dissemination of ovarian cancer cells by inhibiting integrin alpha5 expression, Am. J. Pathol. 182 (2013) 1876–1889.
[36] A. Bonauer, S. Dimmeler, The microRNA-17-92 cluster: still a miRacle?, Cell Cycle 8 (2009) 3866–3873
[37] B.H. Jiang, L.Z. Liu, PI3K/PTEN signaling in angiogenesis and tumorigenesis, Adv. Cancer Res. 102 (2009) 19–65.
[38] K. Utispan, J. Sonongbua, P. Thuwajit, S. Chau-In, C. Pairojkul, S. Wongkham, et al., Periostin activates integrin alpha5beta1 through a PI3K/AKTdependent pathway in invasion of cholangiocarcinoma, Int. J. Oncol. 41 (2012) 1110–1118.