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MicroRNA-27b plays a role in pulmonary arterial hypertension by modulating peroxisome proliferator-activated receptor γ dependent Hsp90-eNOS signaling and nitric oxide production

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ABSTRACT

Pulmonary artery endothelial dysfunction is associated with pulmonary arterial hypertension (PAH). Based on recent studies showing that microRNA (miR)-27b is aberrantly expressed in PAH, we hypothesized that miR-27b may contribute to pulmonary endothelial dysfunction and vascular remodeling in PAH. The effect of miR-27b on pulmonary endothelial dysfunction and the underlying mechanism were investigated in human pulmonary artery endothelial cells (HPAECs) *in vitro* and in a monocrotaline (MCT)-induced model of PAH *in vivo*. miR-27b expression was upregulated in MCT-induced PAH and inversely correlated with the levels of peroxisome proliferator-activated receptor (PPAR)-γ, and miR-27b inhibition attenuated MCT-induced endothelial dysfunction and remodeling and prevented PAH associated right ventricular hypertrophy and systolic pressure in rats. PPARγ was confirmed as a direct target of miR-27b in HPAECs and shown to mediate the effect of miR-27b on the disruption of endothelial nitric oxide synthase (eNOS) coupling to Hsp90 and the suppression of NO production associated with the PAH phenotype. We showed that miR-27b plays a role endothelial function and NO release and elucidated a potential mechanism by which miR-27b regulates Hsp90-eNOS and NO signaling by modulating PPARγ expression, providing potential therapeutic targets for the treatment of PAH.

1. Introduction

Pulmonary arterial hypertension (PAH) is a rare but severe disease characterized by sustained vasoconstriction and progressive narrowing of small resistance pulmonary arteries and arterioles caused by medial thickening, fibrosis and the formation of angioproliferative lesions [1]. Pulmonary artery endothelial dysfunction and apoptosis of pulmonary artery endothelial cells play an early role in the pathogenesis of PAH, whereas the subsequent proliferation and migration of smooth muscle cells, fibroblasts and endothelial cells drive vascular remodeling, which leads to a progressive increase in vascular resistance [2]. Research efforts in the last years have provided insight into the pathogenesis of PAH, and several potential mechanisms have been identified, including alterations of growth factor pathways, cytokines, inflammation, and mitochondrial metabolism, and mutations in the bone morphogenetic protein receptor-2 (BMPR2) gene among others [3]. Emerging evidence indicates that peroxisome proliferator-activated receptor (PPAR)-γ, a ligand-activated transcription factor involved in the regulation of cellular differentiation, development, metabolism, inflammation and tumorigenesis, favorably affects several pathways associated with the pathogenesis of PAH [4]. PPARs heterodimerize with the retinoid X receptor and bind to PPAR response elements in the promoter region of target genes [5]. Endothelial cell PPAR-γ activation downregulates endothelin 1 and the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine, which are involved in insulin resistance and PAH [6,7]. PPAR-γ promotes endothelial cell proliferation and migration by inducing endothelial nitric oxide synthase (eNOS), which produces the vasodilator nitric oxide (NO) from the amino acid L-arginine in endothelial cells. Endothelial dysfunction is characterized by impaired endothelial NO production, and PPAR-γ stimulates the release of NO from the endothelium to protect the vascular wall, suggesting that impaired activation of PPAR-γ targets may be associated with the pathogenesis of PAH [8–10]. eNOS forms a complex with the chaperone

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HSP90, which stabilizes and preserves the activity of the enzyme, thus playing a role in the regulation of NO production in endothelial cells [11].

microRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by specific binding to the 3′-untranslated region (3′-UTR) of target mRNAs, modulating expression by translational repression or mRNA destabilization [12]. Several aberrantly expressed miRNAs have been associated with PAH [13]. The miR-27 family has been shown to play a role in many cellular processes. miR-27b functions as an angiogenic switch by promoting endothelial tip cell fate and sprouting [14,15]. The regulation of mRNAs by the miR-27 family is important in many cancers [16,17]. miR-27b was previously shown to target PPAR-γ in human multipotent adipose-derived stem cells, and it functions as a tumor suppressor in neuroblastoma by downregulating PPAR-γ [18,19].

In this study, we used human pulmonary artery endothelial cells (HPAECs) and an MCT model of pulmonary hypertension to examine the role of miR-27b in pulmonary artery endothelial dysfunction and explore the underlying mechanisms. We show that miR-27b plays a role in pulmonary artery endothelial function and PAH by modulating eNOS activity and NO signaling via its target PPAR-γ, providing insight into the regulation of pulmonary hypertension by miRNAs and potential therapeutic targets for the treatment of PAH.

2. Materials and methods

2.1. Animals and experimental design

All animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of medicine. Adult male Sprague–Dawley rats, 300–350 g in body weight, were treated by subcutaneous injection of saline or 60 mg/kg MCT for 28 days to induce pulmonary hypertension. Rats were divided into two groups, normal control (ctrl, n = 8) and MCT-PAH (n = 36); MCT-PAH rats (n = 36) were randomized into 3 groups and received intravenous injection (tail vein) of PBS, anti-miR-27b, or PPARγ 2 weeks after MCT induction.

2.2. Hemodynamic and morphometric analyses

To measure right ventricular systolic pressure (RVSP), a catheter was inserted into the right jugular vein under isoflurane anesthesia. For calculation of right ventricle to left ventricle plus septum [RV/(LV + S)] ratio, the hearts of rats were dissected and the RV wall was cut out and weighed, then the LV and septum were weighed and the ratio was calculated as an index of RV hypertrophy.

2.3. Morphometric analysis of pulmonary hypertension

Sections of paraffin-embedded lungs were prepared and stained with hematoxylin and eosin (H&E). The slides were evaluated by light microscopy, and the extent of vascular remodeling was assessed by a researcher blinded to the treatment groups.

2.4. Endothelial cell culture

HPAECs were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China) and grown in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2.

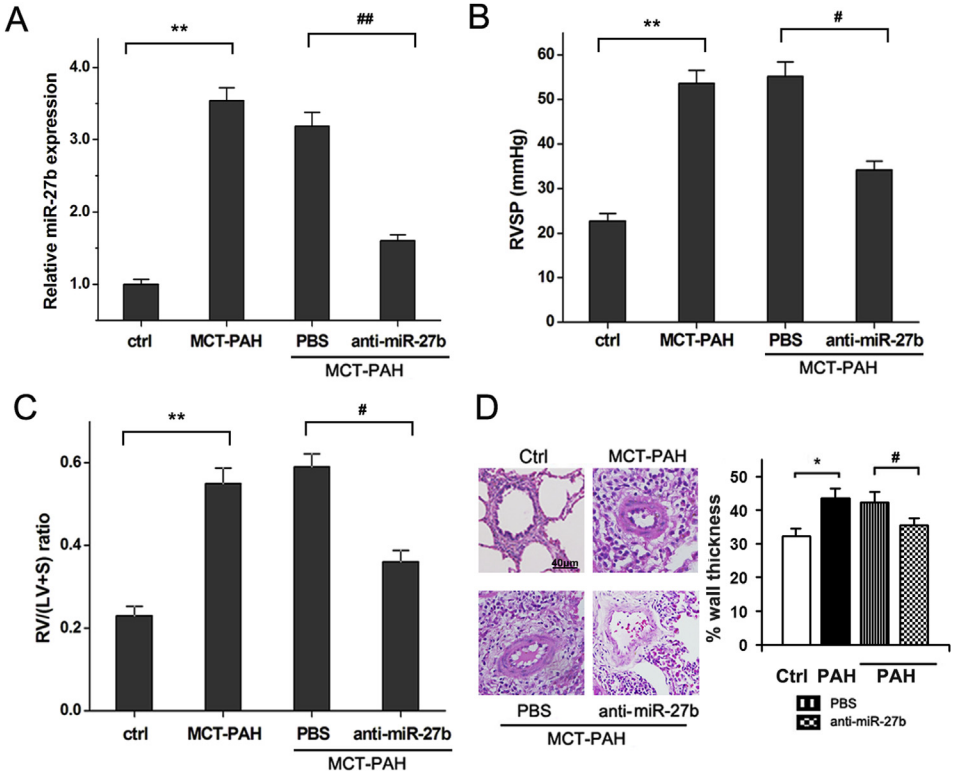

Fig. 1. Effect of miR-27b inhibition on MCT-induced endothelial dysfunction and PAH. (A) RT-PCR analysis of miR-27b expression in the lungs of control (n = 8) and MCT-induced PAH rats in the presence or absence of anti-miR-27b injection (n = 12 per group). (B and C) Effect of miR-27b inhibition on the MCT-PAH induced increase in right ventricular systolic pressure (RVSP) (B) and right ventricular weight to left ventricular plus septal weight ratio RV/(LV + S) (C) (n = 6). (D) Effect of miR-27b inhibition on pulmonary artery remodeling was examined. Representative sections of paraffin-embedded lungs and pulmonary artery percent wall thickness are shown. Scale bar, 40 μm. Values represent the mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. PBS control in MCT-PAH group.

2.5. Immunoprecipitation and immunoblot analysis

For western blot analysis, lysates of HPAECs or lung tissues were prepared in ice cold lysis buffer with protease inhibitors (Pierce Laboratories, Rockford, IL) and subjected to western blot using the indicated primary antibodies and the appropriate horseradish peroxidase (HRP) conjugated secondary antibodies (Abcam, Cambridge, UK). Bands were visualized by enhanced chemiluminescence and normalized to the β-actin loading control. For Immunoprecipitation analysis, proteins were incubated with anti-eNOS or anti-Hsp90 antibody (Abcam) followed by incubation with protein G plus/protein A agarose beads (Calbiochem, La Jolla, CA). After centrifugation, precipitates were immunoblotted for Hsp90 and eNOS following standard protocols.

2.6. Quantitative RT-PCR (qRT-PCR)

Total RNA was extracted using Trizol reagent (Invitrogen, USA). The relative expressions of miR-27b were measured TaqMan microRNA assay (Applied Biosystems) according to the manufacturer's protocol, and snRNA U6 was used as an endogenous control. The relative mRNA expressions were detected by SYBR green qPCR assay (BioRad, Hercules, USA) as previously described [20] and normalized to β-actin. Primers used are as follows: PPARγ, 5′- GGA TGC AAG GGT TTC TTC CG -3′ (forward), 5′- GCA CGT GTT CCG TGA CAA TC -3′ (reverse); β-actin, 5′- GAG CAC AGA GCC TCG CCT TT -3′ (forward), 5′- AGA GGC GTA CAG GGA TAG CA -3′ (reverse).

2.7. Luciferase assays

Firefly luciferase reporter plasmids containing the wild-type 3′-UTR of PPARγ or a mutant derivative bearing mutations in the miR-27b seed sequence were co-transfected with miR-27b, anti-miR-27b, or negative control (NC) into HPAECs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). The ratio of firefly to Renilla luciferase activity was measured using the Dual Luciferase Reporter Assay System (Promega) and expressed as relative to the activity of the NC.

2.8. Measurement of NO level

The levels of NO was determined by measuring ·NO and its oxidation products NO_2^- and NO_3^- (collectively referred to as NOx) in the culture medium above confluent PAEC monolayers and lung tissue homogenate as described previously [21]. NO was detected with a Sievers chemiluminescence nitric oxide analyzer (Model 280; Sievers, Boulder, USA). Standard curves with $NaNO_3$ were performed daily.

2.9. Statistical analyses

Each experiment was repeated at least three times. Data are presented as the mean ± SD. The results were analyzed by a two-tailed Student's *t*-test. The correlation between miR-27b and PPARγ expression were analyzed using Pearson correlation coefficient. A value of $p < 0.05$ was considered to be statistically significant.

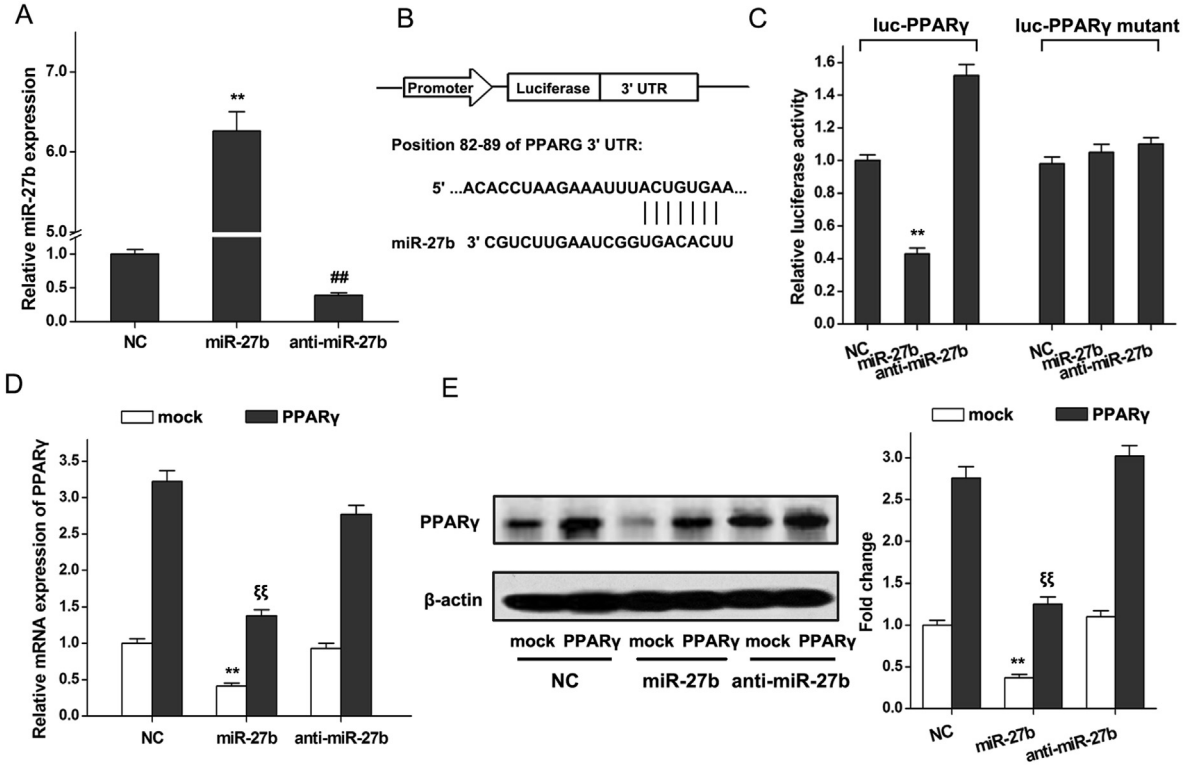

Fig. 2. PPAR-γ is a target of miR-27b in human pulmonary artery endothelial cells (HPAECs). (A) RT-PCR analysis of miR-27b expression in adenovirus infected HPAECs. (B) Conservation of the miR-27b binding site in the 3′-UTR of PPARγ. The miR-27b seed match is indicated by vertical lines. (C) Luciferase activity of reporters containing the wild-type or mutant 3′-UTR (8-bp deleted) of PPARγ 24 h after transfection with miR-27b, anti-miR-27b or negative control (NC). (D and E) PPARγ mRNA (D) and protein (E) levels were assessed by qRT-PCR and western blotting with β-actin as a loading control in HPAECs co-transfected with miR-27b, anti-miR-27b or negative control (NC) and mock or PPARγ expression vector. The results of quantitative analysis of changes in the expression of PPAR-γ are shown. Values represent the mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. NC; $^{\#\#}p < 0.01$ vs. miR-27b group; $^{\S\S}p < 0.01$ vs. NC with PPARγ treatment group.

3. Results

3.1. miR-27b inhibition ameliorates MCT-induced endothelial dysfunction and PAH

To examine the role of miR-27b in endothelial dysfunction leading to pulmonary hypertension, we used a rat model of MCT-induced PAH. miR-27b expression was upregulated in the lungs of MCT-PAH rats compared to untreated controls, and effectively downregulated by anti-miR-27b, (Fig. 1A). Anti-miR-27b significantly attenuated the MCT-PAH induced increases in RVSP and the RV/(LV + S) ratio, which is an indicator of RV hypertrophy (Fig. 1 B and C). Fig 1D shows representative H&E stained sections of rat lungs, indicating pulmonary vascular media hypertrophy induced by MCT, which is attenuated by treatment with anti-miR-27b. Measurement of wall thickness showed the significant effect of miR-27b on attenuating MCT-induced pulmonary artery remodeling.

3.2. miR-27b targets PPARγ in HPAECs

Infection with adenovirus overexpressing miR-27b effectively upregulated and anti-miR-27b downregulated the expression of miR-27b in HPAECs (Fig. 2A). To confirm that PPARγ is a target of miR-27b in HPAECs, luciferase reporter constructs were generated by cloning the wild-type or mutated 3′-UTR region of PPARγ containing the miR-27b seed sequence (Fig. 2B) and co-transfecting them with miR-27b or anti-miR-27b into HPAECs. The results of luciferase assays showed that miR-27b overexpression significantly inhibited and anti-miR-27b significantly increased the reporter activity of the wild-type but not that of the mutant PPARγ 3′-UTR (Fig. 2C). In addition, ectopic expression of miR-27b significantly downregulated PPARγ at the mRNA and protein levels, whereas anti-miR-27b restored PPARγ levels in cells co-transfected with mock or a PPARγ expression vector (Fig. 2D). Taken together, these results indicate that miR-27b directly targets and modulates the expression of PPARγ in HPAECs.

3.3. miR-27b modulates PPARγ dependent Hsp90-eNOS signaling in HPAECs

Based on previous studies showing that PPARγ improves endothelial function by upregulating eNOS activity and NO synthesis [22,23], and the role of Hsp90 on stabilizing eNOS, we examined the effect of miR-27b on PPARγ-dependent eNOS/Hsp90 levels and interaction in HPAECs. Ectopic expression or inhibition of miR-27b did not significantly affect the protein levels of eNOS or Hsp90 in mock or PPARγ overexpressing HPAECs (Fig. 3A and B). Immunoprecipitation analyses showed that miR-27b disrupted the eNOS/Hsp90 interaction, whereas anti-miR-27b or PPARγ overexpression restored eNOS/Hsp90 complex formation in HPAECs (Fig. 3C). miR-27b significantly suppressed NO release compared to the negative control, whereas anti-miR-27b or PPARγ overexpression restored NO levels in HPAECs (Fig. 3D). Taken together, these results indicate that miR-27b disrupts eNOS stability and activity and inhibits endothelial cell NO production via a PPARγ dependent mechanism.

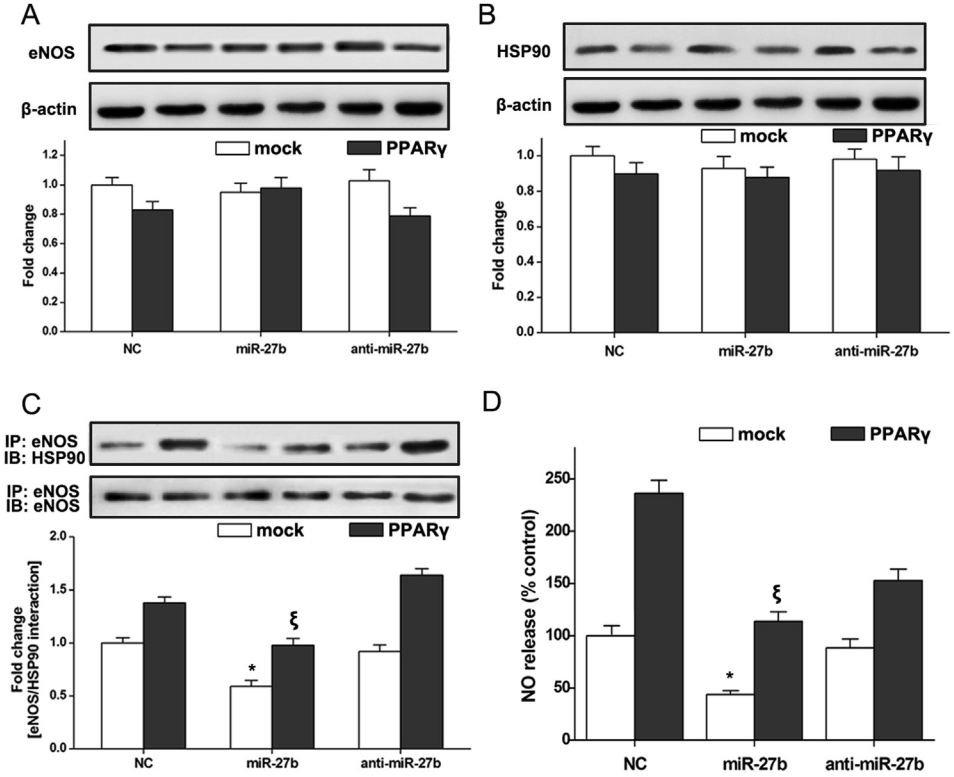

Fig. 3. miR-27b and PPARγ regulate eNOS/Hsp90 interactions and NO production in HPAECs. (A and B) Representative immunoblots of eNOS (A) and HSP90 (B) in HPAECs transfected with the indicated RNAs. The results of quantitative analysis of fold change are showed below. β-actin served as the loading control. (C) Immunoprecipitation analysis of the association between eNOS and Hsp90 in HPAECs in response to miR-27b overexpression or inhibition. Representative immunoblots and the mean eNOS/HSP90 ratio are shown. (D) NO levels were determined in HPAECs transfected with miR-27b, anti-miR-27b or negative control (NC) with mock or PPARγ expression vector. Values represent the mean ± SD (n = 3). $^{*}p < 0.05$, $^{**}p < 0.01$ vs. NC; $^{\xi}p < 0.05$ vs. NC with PPARγ treatment group.

3.4. miR-27b modulates PPARγ dependent Hsp90-eNOS signaling in MCT-induced PAH

To further examine the role of miR-27b in pulmonary artery endothelial function associated with PAH, the effect of miR-27b inhibition on PPARγ expression, eNOS/HSP90 interaction and NO release was assessed in rat lungs exposed to MCT-induced PAH. MCT-PAH significantly downregulated PPARγ, whereas miR-27b inhibition or PPARγ overexpression significantly rescued the mRNA and protein levels of PPARγ in rat lungs (Fig. 4A and B). Correlation analysis showed that the expression of miR-27b was inversely correlated with that of PPARγ in MCT-induced PAH rat lungs ($r = -0.376$, $p < 0.05$; Fig. 4C). Immunoprecipitation analysis further showed that miR-27b inhibition or PPARγ overexpression significantly restored the interaction of eNOS with HSP90, which was significantly decreased by MCT treatment in rat lungs (Fig. 4D). Similarly, the levels of NOx were decreased in response to MCT-induced PAH in rat lungs, and infection with anti-miR-27b or ectopic expression of PPARγ restored NOx to control levels (Fig. 4E). Taken together, these results confirm that miR-27b affects NO production by regulating eNOS/Hsp90 interaction via the modulation of PPARγ expression in the lungs of MCT-PAH rats.

4. Discussion

Endothelial dysfunction plays a critical role in the pathogenesis of pulmonary vascular disease, however, the mechanisms underlying these changes remain unclear. Here, we examined the miR-27b mediated regulation of PPARγ dependent eNOS activity and NO signaling and its role in pulmonary artery endothelial function and PAH. In a rat model of MCT induced PAH, miR-27b was upregulated significantly, and its inhibition ameliorated the PAH phenotype, decreasing RSVP and attenuating right ventricular and pulmonary vascular media hypertrophy. The involvement of miRNAs in the etiology of PAH was suggested by Brock et al., who showed that the miR-17/92 cluster plays a role in the development of pulmonary hypertension by modulating the expression of BMPR2 [24]. Several miRNAs including miR-27b have been shown to play a role in vascular disorders. miR-27b overexpression promotes the hypertrophic growth of primary cultured cardiomyocytes by targeting PPARγ, and administration of anti-miR-27b attenuates cardiac hypertrophy and dysfunction in a mouse model of transverse aortic constriction [25]. miR-27b targets PPARγ to regulate adipocyte differentiation and modulate inflammatory responses [26,27]. In the present study, we analyzed the mechanism by which miR-27b contributes to vascular remodeling in PAH via its target PPARγ. We confirmed PPARγ as a target of miR-27b in HPAECs and showed that miR-27b promotes endothelial dysfunction by downregulating PPARγ. PPARγ plays a role in several diseases including diabetes, inflammation, cancer and atherosclerosis, and its involvement in the progression of pulmonary hypertension has been demonstrated in several studies [27]. Aberrant expression of PPARγ is associated with pulmonary hypertension and contributes to the abnormal endothelial cell phenotype associated with PAH [28,29]. PPARγ mRNA and protein expression are decreased in patients with pulmonary hypertension [26,29], and in the mouse lung, rosiglitazone, a PPARγ antagonist, attenuates hypoxia induced pulmonary hypertension, vascular remodeling and reactive oxygen species generation [30].

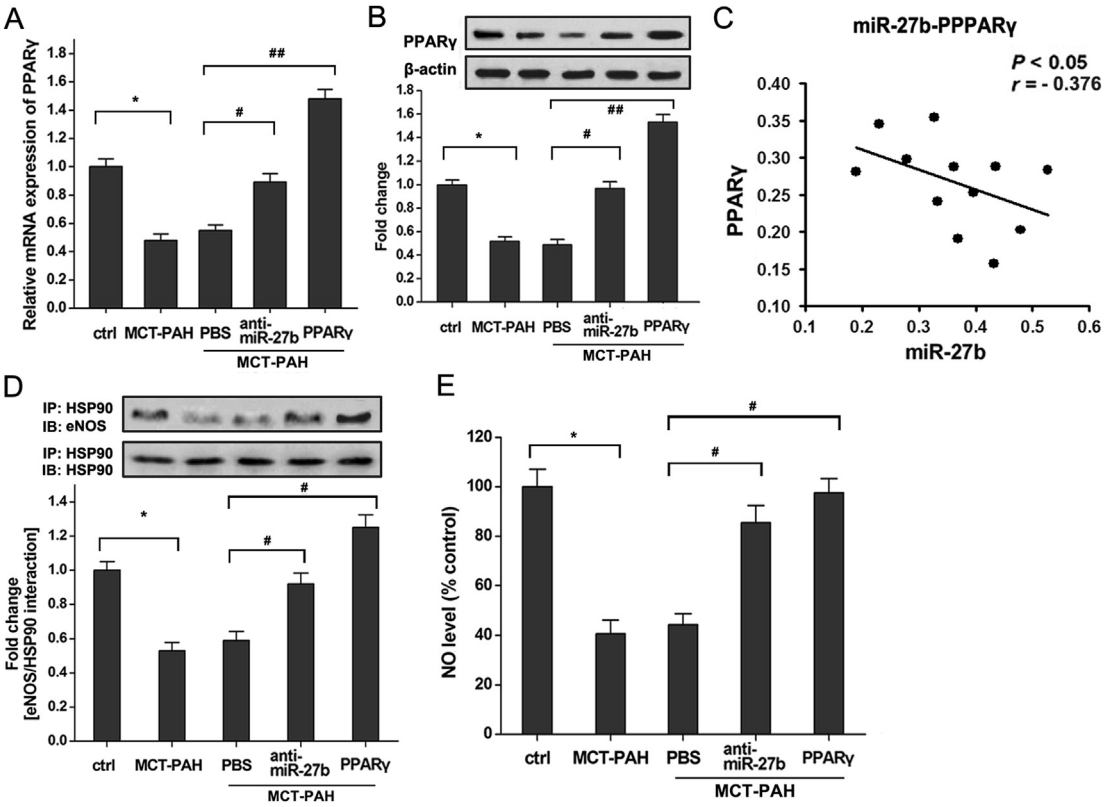

Fig. 4. Effect of miR-27b inhibition on the PPARγ dependent eNOS/Hsp90 interaction and NO production in MCT- PAH. (A and B) PPARγ mRNA (A) and protein (B) levels in the lungs of MCT-induced PAH rats in the presence or absence of anti-miR-27b or PPARγ overexpression (n = 12 per group). (C) qRT-PCR analysis of the correlation between PPARγ and miR-27b expression in MCT-induced PAH rat lungs. (D) Immunoprecipitation analysis eNOS/Hsp90 interaction in rat lungs with the indicated treatments. The levels of eNOS protein associated with Hsp90 relative to total Hsp90 protein were calculated. (E) NOx levels in rat lungs with the indicated treatments. Values represent the mean ± SD. $^{*}p < 0.05$ vs control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. PBS control in MCT-PAH group.

These studies indicate that loss of PPARγ signaling plays an important role in the pathogenesis of PAH, underscoring the importance of our findings showing the miR-27b regulation of PPARγ expression and its role in vascular endothelial cell function.

PPARγ exerts a protective effect on pulmonary vascular function via a number of mechanisms associated with the inhibition of pulmonary vascular remodeling and the promotion of endothelial cell function [31]. PPARγ was shown to stimulate the release of NO, a critical modulator of vascular tone and smooth muscle cell proliferation, from endothelial cells without affecting the expression of eNOS [32,33]. The activity of eNOS is regulated by several factors including phosphorylation, cofactors such as tetrahydrobiopterin (BH_4), FAD, FMN, L-arginine, and oxygen, and protein–protein interactions [34]. Because Hsp90 binds to eNOS and modulates its activity, playing a critical role in the regulation of NO release and consequently the endothelium-dependent responses of blood vessels [11], we examined the effect of the miR-27b/PPARγ axis on Hsp90/eNOS levels and interaction and NO signaling in HPAECs and in our MCT-PAH model. Our results showed that miR-27b did not affect eNOS levels, but disrupted eNOS/Hsp90 interaction and NO production in correlation with the downregulation of PPARγ in both cultured cells and a rat model of PAH. These results indicate that the effect of miR-27b and PPARγ on NO production does not involve the regulation of eNOS expression, but rather the modulation of its activity via coupling to Hsp90. Our results support previous findings showing the involvement of PPARγ in pulmonary vascular disease through the regulation of NO production, and elucidate a potential mechanism underlying the role of miR-27b in vascular disorders.

In conclusion, our findings show that miR-27b upregulation plays a role in the pathogenesis of PAH via the modulation of the expression of its target PPARγ. We elucidated a novel mechanism by which miR-27b regulates the stability of eNOS and NO production through the modulation of PPARγ levels and thus by indirectly affecting the eNOS/Hsp90 interaction. Future studies should further examine miR-27b expression and its correlation with PPARγ levels in PAH, as well as the effect of miR-27b inhibition on other PPARγ mediated pathways such as the oxidative stress response, which may clarify the potential of miR-27b as a therapeutic target for the treatment of vascular disorders.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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References

[1] R.M. Tuder, S.L. Archer, P. Dorfmuller, S.C. Erzurum, C. Guignabert, E. Michelakis, M. Rabinovitch, R. Schermuly, K.R. Stenmark, N.W. Morrell, Relevant issues in the pathology and pathobiology of pulmonary hypertension, J. Am. Coll. Cardiol. 62 (2013) D4–D12.
[2] G. Hansmann, V.A. de Jesus Perez, T.P. Alastalo, C.M. Alvira, C. Guignabert, J.M. Bekker, S. Schellong, T. Urashima, L. Wang, N.W. Morrell, M. Rabinovitch, An antiproliferative BMP-2/PPARgamma/apoE axis in human and murine SMCs and its role in pulmonary hypertension, J. Clin. Invest 118 (2008) 1846–1857.
[3] R.T. Schermuly, H.A. Ghofrani, M.R. Wilkins, F. Grimminger, Mechanisms of disease: pulmonary arterial hypertension, Nat. Rev. Cardiol. 8 (2011) 443–455.
[4] D. Zhang, G. Wang, D. Han, Y. Zhang, J. Xu, J. Lu, S. Li, X. Xie, L. Liu, L. Dong, M. Li, Activation of PPAR-gamma ameliorates pulmonary arterial hypertension via inducing heme oxygenase-1 and p21(WAF1): an in vivo study in rats, Life Sci. 98 (2013) 39–43.
[5] N. Marx, H. Duez, J.C. Fruchart, B. Staels, Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells, Circ. Res. 94 (2004) 1168–1178.
[6] J.T. Kielstein, S.M. Bode-Boger, G. Hesse, J. Martens-Lobenhoffer, A. Takacs, D. Fliser, M.M. Hoeper, Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1414–1418.
[7] F. Martin-Nizard, C. Furman, P. Delerive, A. Kandoussi, J.C. Fruchart, B. Staels, P. Duriez, Peroxisome proliferator-activated receptor activators inhibit oxidized low-density lipoprotein-induced endothelin-1 secretion in endothelial cells, J. Cardiovasc Pharmacol. 40 (2002) 822–831.
[8] P. Balakumar, S. Kathuria, Submaximal PPARgamma activation and endothelial dysfunction: new perspectives for the management of cardiovascular disorders, Br. J. Pharmacol. 166 (2012) 1981–1992.
[9] D.H. Cho, Y.J. Choi, S.A. Jo, I. Jo, Nitric oxide production and regulation of endothelial nitric-oxide synthase phosphorylation by prolonged treatment with troglitazone: evidence for involvement of peroxisome proliferator-activated receptor (PPAR) gamma-dependent and PPARgamma-independent signaling pathways, J. Biol. Chem. 279 (2004) 2499–2506.
[10] C. Gensch, Y.P. Clever, C. Werner, M. Hanhoun, M. Bohm, U. Laufs, The PPARgamma agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells, Atherosclerosis 192 (2007) 67–74.
[11] G. Garcia-Cardena, R. Fan, V. Shah, R. Sorrentino, G. Cirino, A. Papapetropoulos, W.C. Sessa, Dynamic activation of endothelial nitric oxide synthase by Hsp90, Nature 392 (1998) 821–824.
[12] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233.
[13] P. Caruso, M.R. MacLean, R. Khanin, J. McClure, E. Soon, M. Southgate, R.A. MacDonald, J.A. Greig, K.E. Robertson, R. Masson, L. Denby, Y. Dempsie, L. Long, N.W. Morrell, A.H. Baker, Dynamic changes in lung microRNA profiles during the development of pulmonary hypertension due to chronic hypoxia and monocrotaline, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 716–723.
[14] D. Biyashev, D. Veliceasa, J. Topczewski, J.M. Topczewska, I. Mizgirev, E. Vinokour, A.L. Reddi, J.D. Licht, S.Y. Revskoy, O.V. Volpert, miR-27b controls venous specification and tip cell fate, Blood 119 (2012) 2679–2687.
[15] C. Urbich, D. Kaluza, T. Fromel, A. Knau, K. Bennewitz, R.A. Boon, A. Bonauer, C. Doebele, J.N. Boeckel, E. Hergenreider, A.M. Zeiher, J. Kroll, I. Fleming, S. Dimmeler, MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A, Blood 119 (2012) 1607–1616.
[16] L. Jin, O. Wessely, E.G. Marcusson, C. Ivan, G.A. Calin, S.K. Alahari, Prooncogenic factors miR-23b and miR-27b are regulated by Her2/Neu, EGF, and TNF-alpha in breast cancer, Cancer Res. 73 (2013) 2884–2896.
[17] J. Ye, X. Wu, D. Wu, P. Wu, C. Ni, Z. Zhang, Z. Chen, F. Qiu, J. Xu, J. Huang, miRNA-27b targets vascular endothelial growth factor C to inhibit tumor progression and angiogenesis in colorectal cancer, PLoS One 8 (2013) e60687.
[18] M. Karbiener, C. Fischer, S. Nowitsch, P. Opriessnig, C. Papak, G. Ailhaud, C. Dani, E.Z. Amri, M. Scheideler, microRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma, Biochem. Biophys. Res. Commun. 390 (2009) 247–251.
[19] J.J. Lee, A. Drakaki, D. Iliopoulos, K. Struhl, MiR-27b targets PPARgamma to inhibit growth, tumor progression and the inflammatory response in neuroblastoma cells, Oncogene 31 (2012) 3818–3825.
[20] X. Chang, Y. Sun, S. Han, W. Zhu, H. Zhang, S. Lian, MiR-203 inhibits melanoma invasive and proliferative abilities by targeting the polycomb group gene BMI1, Biochem. Biophys. Res. Commun. 456 (2015) 361–366.
[21] M.P. Gupta, M.D. Ober, C. Patterson, M. Al-Hassani, V. Natarajan, C.M. Hart, Nitric oxide attenuates H(2)O(2)-induced endothelial barrier dysfunction: mechanisms of protection, Am. J. Physiol. Lung Cell. Mol. Physiol. 280 (2001) L116–L126.
[22] A.T. Gonon, A. Bulhak, F. Labruto, P.O. Sjoquist, J. Pernow, Cardioprotection mediated by rosiglitazone, a peroxisome proliferator-activated receptor gamma ligand, in relation to nitric oxide, Basic Res. Cardiol. 102 (2007) 80–89.
[23] N. Kobayashi, T. Ohno, K. Yoshida, H. Fukushima, Y. Mamada, M. Nomura, H. Hirata, Y. Machida, M. Shinoda, N. Suzuki, H. Matsuoka, Cardioprotective mechanism of telmisartan via PPAR-gamma-eNOS pathway in dahl salt-sensitive hypertensive rats, Am. J. Hypertens. 21 (2008) 576–581.
[24] M. Brock, M. Trenkmann, R.E. Gay, B.A. Michel, S. Gay, M. Fischler, S. Ulrich, R. Speich, L.C. Huber, Interleukin-6 modulates the expression of the bone morphogenic protein receptor type II through a novel STAT3-microRNA cluster 17/92 pathway, Circ. Res. 104 (2009) 1184–1191.
[25] J. Wang, Y. Song, Y. Zhang, H. Xiao, Q. Sun, N. Hou, S. Guo, Y. Wang, K. Fan, D. Zhan, L. Zha, Y. Cao, Z. Li, X. Cheng, X. Yang, Cardiomyocyte overexpression of miR-27b induces cardiac hypertrophy and dysfunction in mice, Cell. Res. 22 (2012) 516–527.
[26] C. Jennewein, A. von Knethen, T. Schmid, B. Brune, MicroRNA-27b contributes to lipopolysaccharide-mediated peroxisome proliferator-activated receptor gamma (PPARgamma) mRNA destabilization, J. Biol. Chem. 285 (2010) 11846–11853.
[27] S. Sharma, X. Sun, R. Rafikov, S. Kumar, Y. Hou, P.E. Oishi, S.A. Datar, G. Raff, J.R. Fineman, S.M. Black, PPAR-gamma regulates carnitine homeostasis and mitochondrial function in a lamb model of increased pulmonary blood flow, PLoS One 7 (2012) e41555.

[28] S. Ameshima, H. Golpon, C.D. Cool, D. Chan, R.W. Vandivier, S.J. Gardai, M. Wick, R.A. Nemenoff, M.W. Geraci, N.F. Voelkel, Peroxisome proliferator-activated receptor gamma (PPARgamma) expression is decreased in pulmonary hypertension and affects endothelial cell growth, Circ. Res. 92 (2003) 1162–1169.
[29] R.E. Nisbet, R.L. Sutliff, C.M. Hart, The role of peroxisome proliferator-activated receptors in pulmonary vascular disease, PPAR Res. 2007 (2007) 18797.
[30] R.E. Nisbet, J.M. Bland, D.J. Kleinhenz, P.O. Mitchell, E.R. Walp, R.L. Sutliff, C.M. Hart, Rosiglitazone attenuates chronic hypoxia-induced pulmonary hypertension in a mouse model, Am. J. Respir. Cell. Mol. Biol. 42 (2010) 482–490.
[31] M. Rabinovitch, PPARgamma and the pathobiology of pulmonary arterial hypertension, Adv. Exp. Med. Biol. 661 (2010) 447–458.
[32] D.S. Calnek, L. Mazzella, S. Roser, J. Roman, C.M. Hart, Peroxisome proliferator-activated receptor gamma ligands increase release of nitric oxide from endothelial cells, Arterioscler. Thromb. Vasc. Biol. 23 (2003) 52–57.
[33] J.A. Polikandriotis, L.J. Mazzella, H.L. Rupnow, C.M. Hart, Peroxisome proliferator-activated receptor gamma ligands stimulate endothelial nitric oxide production through distinct peroxisome proliferator-activated receptor gamma-dependent mechanisms, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 1810–1816.
[34] N. Sud, S. Sharma, D.A. Wiseman, C. Harmon, S. Kumar, R.C. Venema, J.R. Fineman, S.M. Black, Nitric oxide and superoxide generation from endothelial NOS: modulation by HSP90, Am. J. Physiol. Lung Cell. Mol. Physiol. 293 (2007) L1444–L1453.