miR-106b-5p modulates acute pulmonary embolism via NOR1 in pulmonary artery smooth muscle cells

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Abstract. Acute pulmonary embolism (APE) is a common cause of acute cardiovascular failure and has a high morbidity and mortality rate. Inhibiting the excessive proliferation and migration of pulmonary artery smooth muscle cells (PASMCs) is a potential treatment strategy following an APE. Various microRNAs (miRNAs/miRs) have been shown to regulate cell proliferation, apoptosis and other physiological processes. However, the specific mechanisms underlying the action of multiple miRNAs are still not understood in APE. In the present study, the role of miR-106b-5p on APE was demonstrated in platelet-derived growth factor (PDGF)-induced PASMCs in vitro and in an APE-mouse model in vivo. The results showed that miR-106b-5p expression was downregulated in PDGF-induced PASMCs and APE mice, and NOR1 levels were upregulated. Proliferating cell nuclear antigen (PCNA) expression levels in cells and proliferation of PASMCs proliferation and migration were reduced following treatment with miR-106b-5p agomiR, and increased following treatment with miR-106b-5p antagomiR. miR-106b-5p targeted the 3’ untranslated region of NOR-1 mRNA and reduced NOR1 expression. NOR1 overexpression reversed the effects of miR-106-5p on PASMCs and of pulmonary vascular remodeling through PDGF-induced PASMCs in an APE mouse model via targeting NOR1. These results expand the understanding of the pathogenesis underlying APE and highlight potential novel therapeutic targets.

Introduction

Acute pulmonary embolism (APE) is a common cause of acute cardiovascular failure and is associated with high morbidity and mortality rates (1,2). The embolism causing obstruction of the pulmonary vasculature usually leads to pulmonary hypertension (PH) and cardiac failure. Studies have demonstrated that pulmonary artery smooth muscle cells (PASMCs) excessively proliferate and migrate to the pulmonary artery intima following APE, which results in reconstruction of the pulmonary vasculature and thus increases pulmonary vascular resistance (3,4).

MicroRNAs (miRNAs/miRs) are ~22 nucleotide-long non-coding RNAs, which serve as post-transcriptional modulators of the expression of target genes through binding to the 3’ untranslated regions (UTRs) of their respective target genes. miRNAs affect mRNA expression levels through inhibiting translation or inducing mRNA degradation (5). Various miRNAs have been shown to regulate cell proliferation, death and other physiological processes. Dysregulated expression of various miRNAs is associated with several diseases, including cardiovascular diseases, such as PH. Studies have shown that abnormal expression of miRNAs contribute to the pathogenesis of hypoxia-induced PH (6), such as miR-31a-5p through targeting of TP53 (7), miR-135a through regulation of bone morphogenetic protein receptor type-2 (BMPR2) levels (8) and miR-17 through targeting of mitofusin-2 (9). Yue et al (10) showed that miR-143/145 promotes hypoxia-induced proliferation and migration of PASMCs, and improves hypoxia-induced PH through targeting ABCA1. Courbon et al (11) demonstrated that miR-204 serves a significant role in decreasing proliferation, vascular remodeling and regulating pulmonary artery blood pressure in PH, through targeting of SHP2 (11). Several miRNAs have been identified as biomarkers for chronic thromboembolic pulmonary hypertension (CTEPH) and APE. miR-759, Let-7d, Let-7b and miR-22 have been demonstrated to modulate fibrinolysis, which contributes to...
the development of CTEPH (12). Let-7d suppresses proliferation of PASMC and Let-7b targets TGFβR1 and endothelin-1 reducing migration of pulmonary artery endothelial cells and PASMCs (13,14). Recent studies have shown that expression of miR-23a, miR-221, miR-27a/b, miR-1233 and miR-28-3p are significantly increased in the plasma of patients with APE compared with healthy individuals, and may thus serve as potential biomarkers for APE (1,15-18). Zhang et al (19) demonstrated that miR-23a controls the proliferation and migration of human PASMCs by targeting BMPR2/Smad1 signaling (19). However, the specific mechanisms of several miRNAs remain to be determined in APE. To further understand the pathophysiological mechanisms underlying APE, additional studies examining the effects of miRNAs on APE required.

Li et al (20) demonstrated that miR-106b-5p binds to the 3′-UTR of Angiopoietin 2 (Angpt2) to induce migration and tube formation of HUVECs, and human cholesteatoma peri-matrix fibroblasts (hCPFs)-exosomes transports miR-106b-5p to endothelial cells and promotes angiogenesis by upregulating expression of Angpt2 (20). miR-106b-5p is pivotal in regulating cell proliferation and migration. Thus, it was hypothesized that miR-106b-5p may be closely associated with excessive proliferation and migration of PASMCs following APE. As a member of the NR4A subfamily of nuclear receptors, NOR-1 activity is sustained at a relatively low levels in healthy vascular endothelial cells and is upregulated when affected by external stimuli (21,22). NOR-1 is an effecter of inflammation, growth factors, lipoproteins and thrombin, that controls the spreading, migration and proliferation of vascular cells (23-26). In the present study, miR-106b-5p was downregulated in PDGF-induced PASMCs and in an APE mouse model. Furthermore, miR-106b-5p targeted the 3′ UTR of NOR1 mRNA. The functional roles of miR-106b-5p in PDGF-induced PASMCs and in an APE mouse model were evaluated and the underlying molecular mechanisms were determined.

**Materials and methods**

**Mouse model of APE.** Male C57BL/6 mice (weighing 20±2 g; n=48), were purchased from the animal center of Xi’an Jiaotong University and kept at 22±2°C with a relative humidity of 40-70%, allowed to freely forage, with a 12-h light/dark cycle and ad libitum access to food and water. All animal experiments were performed according to the National Institutes of Health’s Guide for the care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Xi’an Jiaotong University. The APE model was established through self-blood coagulum, as previously described (27). Briefly, 100 µl blood was drawn from the tail veins of the mice. After coagulation, blood was clotted in a 37°C water bath for 30 min and diced into 1x1 mm sections. A total of 30 autologous blood clots were injected followed by 0.4 ml saline to establish the model. In the sham group, mice were injected with 0.4 ml saline. Mice were, respectively, treated with 10 mg/kg agomiR-106b-5p (5′-UAA AGU GcU GUc AcU GUc AcG U-3′) for 3 consecutive days (Guangzhou RiboBio Co., Ltd.) through tail vein intravenous injections, beginning 15 min after establishment of the model. Following the operation, mice were given ad libitum access to water and food. The mortality of the mice in each group was monitored. After 7 days of treatment, mice were euthanized by carbon dioxide asphyxiation (flow rate displacing no more than 30% of the chamber volume/minute, mice were kept in carbon dioxide asphyxiation for 2-3 min, followed by respiratory and cardiac arrest for another 1 min in the box), and lung tissue was obtained to analyze the lung index: Lung index = lung weight (mg)/body weight (g) x100%; and to perform subsequent experiments.

**PDGF-induced PASMCs model.** Mouse PASMCs were purchased from ScienCell Research Laboratories, Inc. PASMCs were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.). Once confluence had reached 80%, PASMCs were treated with 10, 20 or 40 ng/ml PDGF (Sigma-Aldrich; Merck KGaA), and then treated with agomiR-106b-5p, antagoniR-106b-5p (5′-UCUGACUGUC AGCACUUAU-3′), agomiR-NC or antagoniR-NC (5′-UUCCGAACUGUCACGCU-3′), respectively, for 24 h.

**Transfection.** NOR1 lentiviral activation particles (cat. no. sc-421926-LAC; Santa Cruz Biotechnology, Inc.) were used to overexpress NOR1. PASMCs at 80% confluence were treated with the lentiviral particles, incubated at 37°C for 6 h and subsequently the media was replaced. PASMCs were further cultured for 48 h before subsequent experiments were performed.

**Cell proliferation assay.** Proliferation of PASMCs was assessed using a Cell Counting Kit-8 (CCK8; Dojindo Molecular Technologies, Inc.), according to the manufacturer's protocol. A total of 3x10⁴ cells/well were plated in 96-well plates. Following treatment, 10 µl CCK8 solution was added to the culture medium and the cultures were incubated for 1-3 h at 37°C with 5% CO₂. Absorbance was measured at 450 nm with a microplate reader (Invitrogen; Thermo Fisher Scientific, Inc.).

**Transwell invasion assays.** Transwell invasion assays were performed using Transwell chambers (8 µm) coated with Matrigel (BD Biosciences; Becton, Dickinson and Company). In the bottom chamber, 750 µl DMEM supplemented with 10% FBS was added and 250 µl cell suspension at a density of 1x10⁵/ml was added to the upper chamber without FBS. After culturing for 24 h, the cells which had not invaded were removed and the remaining cells were fixed using 75% alcohol for 10 min at room temperature, stained with 0.5% crystal violet for 5 min at room temperature, and imaged in four randomly selected fields under an inverted optical light microscope (magnification, x10). The number of invaded cells in each group was counted.

**Reverse transcription-quantitative (RT-q)PCR.** Total RNA was obtained from the PASMC of lung tissue samples and cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RT was performed in a 20 µl mixture containing 1 µg total RNA using a miRcute Plus miRNA First-Strand cDNA kit (Tiangen Biotech Co. Ltd.) or QuantScript RT kit (Tiagen
Biotech Co. Ltd.) according to the manufacturer's protocols (37.0°C for 15 min, 95.0°C for 5 sec and 4.0°C for 60 min). qPCR experiments were performed using a miRcute Plus miRNA qPCR kit (Tiangen Biotech Co. Ltd.) or a SuperReal PreMix Plus (SYBR Green) (Tiangen Biotech Co. Ltd.) according to the manufacturer's protocols (95°C for 2 min, followed by 35 cycles of 95°C for 15 sec, 55°C for 30 sec and 72°C for 10 sec; final elongation at 72°C for 10 min). U6 and GAPDH were used as internal controls for miRNA and mRNA, respectively. The sequences of the primers used were: NOR1 forward, 5'-TGCTTTCGTTCTAGACGACG-3' and reverse, 5'-GTCCTCGTCCACTGATGTAT-3'; GAPDH forward, 5'-AACCCTCCATGCTGGAAG-3' and reverse, 5'-GGATCGGGATGATGTCTT-3'; miR-106b-5p, 5'-UAA AGUGCUAGACGUCAAGAU-3'; and U6, 5'-CAAATTCGTTAGCGTTCATAT-3'. Relative gene expression data were analyzed using reverse transcription-quantitative PCR and the 2^(-ΔΔCt) method (28). The level of mRNA was determined by calculating the intensity ratio of NOR1 mRNA/GAPDH mRNA or miR-106b-5p/U6.

**Western blotting.** Total proteins were obtained from the PASMCs of lung tissue samples and cells using RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein concentrations were quantified using a bicinchoninic acid protein kit (Invitrogen; Thermo Fisher Scientific, Inc.). Protein (40 µg/lane) was loaded onto 10% SDS-PAGE gels. Following SDS-PAGE and transfer to a PVDF membrane (EMD Millipore), membranes were blocked in 5% BSA (Sigma-Aldrich; Merck KGaA). Membranes were incubated with the primary antibodies at 4°C overnight. The primary antibodies used were anti-NOR1 (cat. no. ab94507; 1:1,000; Abcam), anti-proliferating cell nuclear antigen (PCNA; cat. no. ab92552; 1:1,000; Abcam) and anti-GAPDH (cat. no. 5174S; 1:2,000; CST Biological Reagents Co., Ltd.). Subsequently, membranes were incubated with the Horseradish peroxidase, Goat Anti-Rabbit IgG secondary antibody (cat. no. A21020; 1:2,000; Abbkine Scientific Co., Ltd.) for 2 h at room temperature. Signals were visualized using Clarity Western ECL Substrate (Bio-Rad Laboratories, Inc.) and detected using a MiVnt image analysis system (Bio-Rad Laboratories, Inc.). Optical densities of the bands were calculated using Image Lab v5.2.1 (Bio-Rad Laboratories, Inc.).

**Luciferase assays.** Bioinformatics analysis using TargetScan v7.2 (http://www.targetscan.org/vert_72/), PicTar (http://www.pictar.org/), and miRanda (http://www.microrna.org/microrna/home.do) predicted that NOR-1 was a potential target gene of miR-106b-5p (29,30). Plasmids containing a portion of wide type (WT) or mutated (Mut) 3'-UTR of the NOR1 gene were synthesized by Guangzhou RiboBio Co., Ltd. A total of 4x10^4 PASMCs/well were plated and co-transfected with 200 ng luciferase vector (Promega Corporation) and 100 nM agomiR-106b-5p or agomiR-NC using Lipofectamine™ 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). After 48 h, PASMCs were lysed and a Dual-Luciferase Reporter Assay (Promega Corporation) was then performed as follows: Luciferase Assay Reagent II was injected, the relative light unit (RLU) of Firefly luciferase activity was measured, Stop and Glo® reagent was injected, the RLU of Renilla luciferase activity was measured and the level of RLU Firefly/RLU Renilla was analyzed according to the manufacturer's protocol.

**RNA immunoprecipitation (RIP).** RIP of miRNA ribonucleoprotein complex with anti-Argonaute 1 (Ago2; Abcam) or immunoglobulin G (IgG; Sigma-Aldrich; Merck KGaA) was performed as previously reported (17). When PASMCs reached 80% confluence, they were transfected with 100 nM agomiR-106b-5p or agomiR-NC for 24 h. Cell lysates were harvested using RIP buffer, incubated with magnetic beads bound with anti-Ago2 or IgG antibodies. After digestion with proteinase K, the precipitated complexes were collected and the RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently RT-qPCR was performed to confirm the miR-106b-5p target was NOR1.

**Statistical analysis.** Data are presented as the mean ± standard error of the mean of four experimental repeats. All statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad Prism Software, Inc.). Differences between groups were compared using a Student's t-test or a one-way ANOVA followed by a post-hoc Tukey's test. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**PDGF reduces miR-106b-5p expression and increases activity of the NOR-1-PCNA signaling pathway in PASMCs.** To examine the roles of miR-106b-5p on PASMCs, miR-106b-5p levels and NOR-1-PCNA pathway activity were detected following treatment with 0, 10, 20 or 40 ng/ml PDGF in PASMCs. As shown in Fig. 1, PDGF significantly increased miR-106b-5p levels, but increased NOR-1 mRNA and PCNA expression levels in a dose-dependent manner. miR-106b-5p levels were decreased 0.75-fold, 0.4-fold and 0.39-fold when treated with 10, 20 and 40 ng/ml PDGF, respectively, compared with the control group (Fig. 1A). NOR-1 mRNA expression levels were increased 1.65-fold, 2.97-fold and 2.63-fold when treated with 10, 20 and 40 ng/ml PDGF treatment, respectively, compared with the control group (Fig. 1B). PCNA expression levels were increased 1.45-fold, 2.07-fold and 2.33-fold when treated with 10, 20 and 40 ng/ml PDGF treatment, respectively, compared with the control group (Fig. 1C). Thus, PDGF treatment reduced miR-106b-5p expression levels and increased proliferation of PASMCs in a dose-dependent manner. For subsequent experiments, 20 ng/ml PDGF was used.

**miR-106b-5p reverses PDGF-induced proliferation and migration of PASMCs.** To determine the effect of miR-106b-5p on the proliferation and migration of PASMCs, agomiR-106b-5p and antagoniR-106b-5p were used to induce or suppress miR-106b-5p expression in PASMCs, respectively, and the levels of miR-106b-5p was assessed using RT-qPCR (Fig. S1A). CCK8 assays were used to assess cell viability. Consistent with previous results, PDGF significantly increased the viability of cells and agomiR-106b-5p inhibited the viability of cells compared with the control group (P<0.05). AgomiR-106b-5p reversed the PDGF-induced increase in viability in PASMCs compared with the PDGF group (P<0.05; Fig. 2A). PCNA
validate this prediction, the specificity of miR-106b-5p to NOR-1 was demonstrated that there was an ~5-fold enrichment of NOR-1 and 3'UTR luciferase reporter assays. The RIP assay results showed that miR-106b-5p negatively regulates NOR-1 expression levels and (c) PcNA protein expression levels following treatment with antagomiR-106b-5p and/or PDGF, and agomiR-106b-5p also suppressed PDGF-induced NOR-1 expression levels (P<0.05; Fig. 4D). Thus, these data demonstrate that miR-106b-5p negatively regulates NOR-1 expression via binding to the 3' UTR.

Activated NOR-1 reduces miR-106b-5p inhibition of PASMC proliferation and migration. After 48 h transfection with NOR1 lentiviral activation particles or NC particles in PASMCs, transfection efficiency for NOR1 overexpression are shown in Fig. S1B and C. NOR1 lentiviral activation particles induced NOR1 mRNA and expression. Then cells were treated with agomiR-106b-5p and/or PDGF, and cell viability and migration were measured. NOR-1 and PDGF significantly increased cell viability and agomiR-106b-5p decreased cell viability, but the effect of agomiR-106b-5p was significantly reduced in NOR-1 activated PASMCs (Fig. 5A). Activated NOR-1 increased cell invasion in the agomiR-106b-5p treatment group, the invasive cell count was 40.7±3.84 and 53.7±2.60 in the agomiR-106b-5p group and the NOR-1 + agomiR-106b-5p group, respectively (Fig. 5B). Western blotting results showed that NOR-1 activation significantly increased PCNA expression in the NOR-1 + agomiR-106b-5p group compared with the agomiR-106b-5p group (P<0.05; Fig. 5C). Thus, NOR-1 activation reversed the inhibition of miR-106b-5p on proliferation and migration of PASMCs.

AgomiR-106b-5p improves APE-induced mortality and increases pulmonary vascular proliferation in mice. An APE mouse model was established for investigating the positive effect of miR-106b-5p in vivo. Following agomiR-106b-5p treatment, some mice were maintained for 15 days to monitor the survival and some of these mice were euthanized on day 7 to assess pulmonary vascular proliferation. Survival of the APE-induced mice after 7 days was 30% and all mice were dead after 12 days. AgomiR-106b-5p reduced the number of early deaths in APE-mice and 40% of mice were still alive at the end of the study (Fig. 6A). Considering the importance of NOR-1 and PCNA in vascular proliferation, the mRNA expression levels of NOR-1 and PCNA were assessed in the pulmonary artery. APE significantly increased the levels of NOR-1 and PCNA in agomiR-106b-5p-treated APE-mice (both P<0.05; Fig. 6B). As shown in the immunohistochemistry results, NOR-1 expression was higher in the narrowed blood vessels of APE-mice and lower in the neointima of pulmonary vasculature in agomiR-106b-5p-treated APE-mice (Fig. 6C). These results suggest that agomiR-106b-5p reduced APE-induced lung injury via inhibition of NOR-1 in the pulmonary vasculature of mice.
Figure 2. AgomiR-106b-5p alleviates PDGF-induced proliferation and migration of PASMCs. PASMCs were treated with 20 ng/ml PDGF and subsequently treated with agomiR-106b-5p. (A) Cell viability was assessed using a Cell Counting Kit-8 assay. (B) Western blotting and densitometry analysis of PCNA expression. (C) Transwell invasion assays were performed to assess invasion of cells following the various treatments. Scale bars: 20 µm. *P<0.05 vs. Ctrl group. #P<0.05 vs. PDGF group. miR, microRNA; PDGF, platelet-derived growth factor; PASMC, pulmonary artery smooth muscle cell; PCNA, proliferating cell nuclear antigen; NC, negative control; Ctrl, control.

Figure 3. AntagomiR-106b-5p increases proliferation and migration of PASMCs. (A) After treatment with PDGF or antagomiR-106b-5p, the viability of PASMCs was assessed using a Cell Counting Kit-8 assay. (B) PCNA protein expression following the various treatments. (C) Transwell invasion assays were performed to assess invasion following the various treatments. Scale bars: 20 µm. *P<0.05 vs. Ctrl group. #P<0.05 vs. PDGF group. miR, microRNA; PDGF, platelet-derived growth factor; PASMC, pulmonary artery smooth muscle cell; PCNA, proliferating cell nuclear antigen; NC, negative control; Ctrl, control.
Numerous studies have shown that the excessive proliferation and migration of PASM cs is involved in the reconstruction of pulmonary vasculature and the increase in pulmonary vascular resistance in APE (3,4). In the present study, it was demonstrated that miR-106b-5p expression was down-regulated in PdGF-induced PASM cs and in an APE mouse model. miR-106b-5p suppressed the proliferation and migration of PASM cs in the APE mouse model and in the PdGF-induced model, by targeting NOR-1. Furthermore, NOR-1 overexpression reversed the reduction of proliferation and migration in miR-106b-5p agomiR-treated PASM cs. AgomiR-106b-5p improved APE-induced mortality and pulmonary vascular proliferation in mice.

As technologies have improved, a large amount of data regarding the expression of various miRNAs in APE have been obtained. Using an Affymetrix miRNA array, Miao et al (27) showed that 24 miRNAs were upregulated and 22 miRNAs were downregulated miRNAs (including miR-3148) when comparing a c TEPH group with control samples, and the number of target genes co-regulated by miR-3148 and other...
miRNAs was the highest of all miRNAs (27). Guo et al. (14) found that miR-1260, miR-602, miR-129-5p, miR-1908 and miR-483-5p were upregulated, and miR-140-3p, miR-93, miR-22, miR-106b and let-7b were downregulated in patients with CTEPH compared with the healthy controls, using a miRCURY LNA Array. As an anti-tumor factor or tumor promoter, miR-106b-5p, the matured product of miR-106b, has been reported to serve an important role in multiple types of cancer. miR-106b-5p targets CTSA to suppress the invasion and metastasis of colorectal cancer (31), but miR-106b-5p promotes stem cell-like properties of hepatocellular carcinoma cells through targeting PTEN via a PI3K/Akt signaling pathway (32). Li et al. (20) demonstrated that miR-106b-5p binds the 3’-UTR of Angpt2 to induce migration and tube formation of HUVECs, and hCPSFs-exosome transport to endothelial cells expressing relatively low amounts of miR-106b-5p, promoting angiogenesis through upregulation of Angpt2 (20). miR-106b-5p is pivotal in regulating cell proliferation and migration. Studies have demonstrated that PASMCs excessively proliferate and migrate to the pulmonary artery intima following APE, resulting in the reconstruction of the pulmonary vasculature and increasing pulmonary vascular resistance (3,4). Thus, it was hypothesized that miR-106b-5p may be closely associated with excessive proliferation and migration of PASMCs following APE. In the present study, the expression of miR-106b-5p in patients with APE and in PDGF-induced PASMCs was determined, and was shown to be decreased compared with the control. In atherosclerotic plaques, Zhang et al. (33) showed that the levels of miR-106b-5p were downregulated compared with normal vascular tissues. TNF-α downregulates miR-106b-5p levels and increases caspase-3 activation and cell DNA fragmentation levels, and upregulation of miR-106b-5p relieves TNF-α-induced apoptosis through targeting PTEN in HUVECs (33). In the present study, it was shown that NOR-1 was a direct target of miR-106b-5p, PDGF treatment increased NOR-1 expression and proliferation of human PASMCs, but miR-106b-5p agomiR reduced proliferation of PASMCs through suppression of NOR-1 expression. miR-106b-5p agomiR alleviated the thrombus-induced intimal hyperplasia and NOR-1 expression in neointimal SMCs of APE mice.

NOR-1 is an effector of inflammation, growth factors, lipoproteins and thrombin, which regulates the migration and proliferation of vascular cells (23-26). PDGF and thrombin induce rapid upregulation of NOR-1 expression, via multiple pathways, including alterations of cytosolic [Ca^{2+}], via the ERK-mitogen associated protein kinase signaling pathway and downstream activation of cAMP response element binding protein (25,34). A previous study demonstrated that NOR1 increases S phase kinase-associated protein 2 expression during the proliferation which underlies neointima formation, which itself is involved in vascular proliferation (35). NOR1 also mediates the expression of several genes and proteins during vascular survival, such as cellular inhibitor of apoptosis 2, Cyclin D1, antiproteinase α-2 macroglobulin, matrix metalloproteinase (MMP)-2 and MMP-9 (36-38). In a guidewire-induced arterial injury mouse model, NOR1 deletion reduced neointima formation during arterial injury (39).

Figure 6. AgomiR-106b-5p alleviates APE-induced lung injury through inhibition of NOR-1 in a mouse model of APE. After treatment with agomiR-106b-5p in the APE mouse model, (A) survival was monitored for 15 days (n=10). (B) Pulmonary vascular proliferation was assessed by measuring the mRNA expression levels of NOR-1 and PCNA protein expression levels in the pulmonary artery and (C) immunohistochemistry analysis of NOR-1 was performed in the pulmonary vasculature after 7 days, n=6. Scale bars: 20 µm. n=10. *P<0.05 vs. Ctrl; †P<0.05 vs. APE group. Ctrl, control; miR, microRNA; PDGF, platelet-derived growth factor; PASMC, pulmonary artery smooth muscle cell; APE, acute pulmonary embolism.
whereas, NOR1-overexpression in SMCs increased mitogenic activity and upregulated expression of embryonic smooth muscle myosin heavy chain, resulting in increased neointima formation in transgenic mice (40). In the present study, NOR1 expression levels were increased in PDGF-induced PASMCs and the neointimal SMCs of APE mice. PASMC viability and intimal hyperplasia were significantly reduced following administration of miR-106-5p agomiR via inhibition of NOR1. Rodríguez-Calvo et al (41) showed that the increased transcriptional activity of Cyclin D2 and increased proliferation in NOR1-overexpressing aortic SMCs and carotid artery ligation resulted in more severe neointimal formation and hemadostenosis in NOR1-transgenic mice compared with the wild-type mice. In the present study it was also shown that NOR-1 overexpression reversed the inhibition of proliferation in miR-106b-5p agomiR-treated PASMCs. Furthermore, NOR1 has been confirmed to be a downstream target of miR-638 (42,43). miR-638 expression was reduced in PDGF-induced human SMCs, in a dose and time-dependent manner. NOR1 is a downstream target gene of miR-638 and NOR1 inhibition is pivotal for miR-638-mediated suppression of PDGF-induced cyclin D1 expression and cell proliferation in human aortic SMCs (42). miR-638 also regulates aberrant proliferation and migration of airway SMCs via targeting of NOR1 and Cyclin D1, which contributes to enhanced airway smooth muscle mass associated with asthma.

In conclusion, the role of miR-106b-5p in APE has not been reported previously, to the best of our knowledge and it was confirmed that miR-106-5p was a novel regulator of PASMC proliferation and pulmonary vascular remodeling via targeting of NOR1. miR-106-5p expression levels were decreased in PDGF-induced PASMCs and overexpression of miR-106-5p decreased proliferation and migration of PASMCs following PDGF stimulation. NOR1 overexpression reversed the effects of miR-106-5p on PDGF-induced PASMCs, suggesting that miR-106-5p serves a significant role in PASMCs through mediating NOR1 activity. Together, these results suggest a novel molecular mechanism underlying proliferation and migration of PASMCs, improving understanding of the pathogenesis of APE and the development of novel therapeutic targets.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

QM, JZ and YM performed the experiments. HC and LP were responsible for analysis and interpretation of the results. HC, LP and HT designed the experiments, and HC wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were performed according to NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Xi’an Jiaotong University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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