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Dysregulation of miR-330-3p is Involved in the Occurrence and Development of Pulmonary Arterial Hypertension Caused by Congenital Heart Disease

ABSTRACT

Background: The study aimed to investigate the expression of miR-330-3p and its clinical and functional performance in congenital heart disease-associated pulmonary hypertension (CHD-PAH).

Methods: The expression of miR-330-3p in CHD-PAH and hypoxia-treated human pulmonary artery smooth muscle cells (HPASMCs) was assessed using reverse transcription quantitative polymerase chain reaction (RT-qPCR). The receiver operating curve was conducted to evaluate the clinical diagnostic value of serum miR-330-3p in CHD-PAH. In cytology, CCK-8 and Transwell migration assays were performed to assess the functional role of miR-330-3p in hypoxia-induced HPASMCs. The online TargetScan database and dual-luciferase reporter assays were employed to explore the downstream target of miR-330-3p.

Results: Compared with healthy controls and patients without PAH, miR-330-3p expression was upregulated in patients with PAH. Serum miR-330-3p expression has relatively high area under the curve (AUC) values in differentiating CHD-PAH patients from congenital heart disease (CHD) patients and healthy individuals. Silencing miR-330-3p weakened the increased cell proliferation, migration, and inflammation caused by hypoxia in HPASMCs. KLF-10 was identified as a putative target of miR-330-3p. Knockdown of KLF-10 could partially reverse the influence of miR-330-3p knockdown in hypoxia-induced HPASMCs.

Conclusion: Upregulation of miR-330-3p might have diagnostic value for predicting individuals suffering from CHD-PAH. Silencing of miR-330-3p reduced the excessive proliferation, migration, and inflammation of hypoxia-exposed HPASMCs by targeting KLF10, which is expected to be a novel small-molecule drug for the targeted treatment of CHD-PAH.

Keywords: Congenital heart disease, pulmonary hypertension, miR-330-3p, KLF10, diagnosis

INTRODUCTION

Approximately 10% of patients with congenital heart disease (CHD) develop pulmonary hypertension (PAH) as a secondary condition, which negatively impacts their quality of life and longevity.[1] Pulmonary hypertension is a group of diseases characterized by the continuous increase of pulmonary artery pressure and pulmonary vascular resistance, deterioration of right heart function, and common comorbidity of body-lung shunt congenital heart disease.[2] The pathogenesis of PAH is usually considered to be an increase in pulmonary vascular resistance (PVR), excessive constriction of the pulmonary vasculature due to sustained endothelial cell function, which results in a loss of balance between vasodilatory and contractile substances, and an aberrant proliferation of pulmonary artery smooth muscle cells (PASMCs), ultimately leading to vascular cell remodeling of the pulmonary arteries.[3] Pulmonary hypertension, as an incurable disease, has a complex pathological process and pathogenesis. Although the academic community has made great progress in understanding and treating this disease in recent years

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and conducted in-depth research on clinical treatment, the current treatment strategies have not yet been able to completely solve all the problems at the root.[4] Further in-depth research on the molecular and cellular mechanisms of its pathogenesis is expected to provide crucial reference information for the development of novel treatment strategies.

microRNAs (miRNAs) are a large family of small non-coding molecules (~22 nucleotides), which exist abundantly in plants, animals, and even viruses. Numerous studies have indicated that miRNAs are associated with cardiac function, development, regeneration, and aging, such as the miR-17-92 cluster, miR-222, and miR-26a.[5-7] microRNAs are increasingly studied to act as potential biomarkers for the diagnosis and treatment of diseases and participate in the pathology and physiology of disease by modulating the expression of target genes by regulating their post-transcriptional levels.[8,9] To date, there are few studies on miRNAs and PAH secondary to CHD. A study revealed that the differential expression of miR-27b (upregulated) and miR-451 (downregulated) was a risk factor for patients with CHD-related PAH.[10] The dynamic changes of miR-21 could modulate right ventricular dysfunction in CHD-associated PAH.[11]

miR-330-3p plays a crucial role in the occurrence and progression of various diseases.[12,13] For instance, miR-330-3p suppresses atherosclerosis by inhibiting cell apoptosis and facilitating proliferation via regulating AQP9.[14] Moreover, the aberrant expression of miR-330-3p could reflect the underlying atrial remodeling progression and arrhythmia recurrence after catheter ablation in patients with atrial fibrillation.[15] A next-generation sequencing study identified abnormal transpulmonary exosomal miRNAs in CHD-related PAH and miR-330-3p was one of the upregulated miRNAs.[16] However, the predictive performance and functional role of miR-330-3p in PAH caused by CHD remain elusive. The current study measured the serum miR-330-3p expression in patients with CHD-related PAH utilizing the quantitative reverse transcription polymerase chain reaction (RT-qPCR) method and its influence on the proliferative capacity and inflammatory factors of pulmonary artery smooth muscle cells.

METHODS

Study Subject's Enrollment

This study included a total of 199 participants, including 50 healthy controls, 60 CHD patients without PAH (simple CHD, named CHD group), and 89 CHD patients with PAH (CHD-PAH, 44 mild cases and 45 severe cases). From 2021 to 2023, CHD patients with ventricular septal defects admitted to the hospital were enrolled in this study. Patients were divided into 2 main groups based on the presence or absence of PAH. The CHD group included 60 patients without PAH [mean pulmonary artery pressure (mPAP) < 25 mm Hg] and the CHD-PAH group consisted of 89 CHD patients with PAH (mPAP ≥ 25 mm Hg). The definition for PAH is based on the Chinese Guidelines for the Diagnosis and Treatment of Pulmonary Hypertension (2021 edition). Among the CHD-associated PAH patients, 44 patients had mild PAH (25 mm Hg ≤ mPAP < 35 mm Hg), and 45 patients had moderate to severe PAH (mPAP ≥ 35 mm Hg). The mean pulmonary arterial pressure (mPAP) was accurately measured via heart catheterization, which is the gold-standard method for assessing pulmonary hemodynamics. The diagnosis of CHD was confirmed by physical examination, electrocardiogram, and cardiac ultrasound. Additionally, diffuse pulmonary alveoli, idiopathic pulmonary hypertension, pneumonia, and pulmonary stenosis were excluded. In addition, 50 healthy individuals were randomly selected from the people who underwent physical examination during the same timeframe to serve as the control group. General information and right cardiac catheterization data were collected and recorded for further analysis.

The study was performed in line with the principles of the Declaration of Helsinki. All experiments are subject to consent by the patients and signed informed consent in accordance with the standards of Ethics Committee of Guizhou Provincial People's Hospital.

Serum Sample Collection

The fasting peripheral venous blood (6 mL) was collected from selected subjects in the early morning using a coagulant tube. Subsequently, the blood specimens were centrifuged at 3000 r/min for 8 minutes to isolate the serum samples. The serum was stored at −80°C for testing.

Cell Culture and Teatment

Human pulmonary artery smooth muscle cells (HPASMCs) were purchased from Wuhan Pricella Biotechnology Co., LTD and cultured in Dulbecco's Modified Eagle's medium (DMEM) medium with 10% fetal bovine serum (FBS) at 37°C. For hypoxic experiments, cells were cultured in an incubator under hypoxia conditions with a supply of 3% O_2, 5% CO_2, and balanced N_2 at 37°C for 24 hours.[17]

MiR-330-3pmimic(5′-GCAAAGCACACGGCCUGCAGAGA-3′), mimic negative control (NC; 5′-GGUUCGUACGUACACUGUUCA-3′), miR-330-3p inhibitor (5′-UCUCUGCAGGCCGUGUGCUUUGC-3′), inhibitor NC (5′-CAGUACUUUUGUGUAGUACAA-3′), KLF10 siRNA (si-KLF10; 5′-UUAUCCUUGAUGAAUCAAUCUGAGG-3′), and siRNA NC (si-NC; 5′-UAACGACGCGACGACGUAATT-3′) were purchased from RiboBio (Guangzhou, China). They were transfected or co-transfected into HPASMCs utilizing Lipofectamine 3000 reagent (Thermo Fisher Scientific, MA, USA) to modulate miR-330-3p or KLF10 expression *in vitro*.

RNA Isolation and RT-qPCR

Total RNA was isolated from serum samples or cells utilizing Trizol reagent (Invitrogen, Carlsbad, CA, USA). The purity and concentration of total RNA (A260/280 nm in the

HIGHLIGHTS

- Serum miR-330-3p was upregulated in patients with CHD-PAH and CHD.
- Serum miR-330-3p may be a putative diagnostic biomarker for CHD-PAH.
- Silencing of miR-330-3p acted a protective role in CHD-PAH by targeting KLF10.

range of 1.8-2.2) were measured using a Nanodrop-2000 UV-Vis spectrometer (Thermo Scientific, MA, USA). The miRNA cDNA synthesis kit (for miR-330-3p) and PrimeScript RT Reagent Kit with genomic DNA (gDNA) eraser (for mRNA; TaKaRa, Dalian, China) were used. The polymerase chain reaction (PCR) amplification was conducted using the ABI750 real-time PCR detection system (Applied Biosystem, Foster City, CA, USA) utilizing SYBR staining. The sequences for PCR were as follows: miR-330-3p, forward, 5′-GCCGAGGCAAAGCACACGGCC-3′, reverse, 5′-CTCAACTGGTGTCGTGGA-3′; U6, forward, 5′-TGGAACGCTTCACGAATTTGCG-3′, reverse: 5′-GGAACGATACAGAGAAGATTAGC-3′; KLF10, forward, 5′-CCAACCATGCTCAACTTCGGTGCCTCT-3′, reverse, 5′-TTCTGACTCTTCACTTTCCGGTCTGTC-3′; GAPDH forward, 5′-AGGTCGGTGTGAACGGATTTG-3′, reverse, 5′-TGTAGACCATGTAGTTGAGGTCA-3′. The expression levels for miR-330-3p or other mRNAs were quantified with the $2^{-\Delta\Delta Ct}$ method using U6 or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for normalization.

Cell Viability Assay

Cell viability was assessed by the Cell Counting Kit 8 (CCK-8; Dojindo, Japan) assay. Hypoxic-treated or transfected HPASMCs were seeded in 96-well plates, and 10 μL CCK-8 kit was added to the wells at certain points in time (0, 24, 48, 72 hours). The cells were further incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 1 hour, and the absorbance (450 nm) was detected to construct the cell proliferation curve.

Cell Migration Assay

The abilities of cell migration were measured using a 24-well Transwell assay (8 μm diameter). The HPASMCs in serum-free medium (2×10^5 cells/well) were added to the top chamber, and the lower chamber was added the complete DMEM medium with 10% FBS as a chemoattractant. After incubating in normoxic or hypoxic environments for 24 hour, the non-migrated cells were removed, and the cells in the lower chamber were fixed with 4% paraformaldehyde, stained with crystal violet, and counted under the microscope in 5 randomly selected fields.

Dual-Luciferase Reporter Assay

The binding sites between KLF10 and miR-330-3p were predicted with the help of the online algorithm TargetScan Human 8.0 (https://www.targetscan.org/). To experimentally validate the predicted binding, 2 types of luciferase reporter plasmids were synthesized by RiboBio Co., Ltd. The first plasmid, denoted as KLF10-WT, contained the wild-type 3′UTR of the KLF10 gene, which harbored the predicted miR-330-3p binding sites. The second plasmid, named KLF10-MUT, was a mutant version where the predicted binding sites in the KLF10 3′UTR were specifically mutated using site-directed mutagenesis techniques. The constructed plasmids (KLF10-WT or KLF10-MUT) were cotransfected with miR-330-3p mimic or mimic NC into HPASMCs with the help of Lipofectamine 3000. Following 48 hours of transfection, the luciferase activity was determined with a luciferase activity assay kit.

Statistical Analysis

Data from 3 independent experiments were analyzed and graphed using GraphPad 9.0 (San Diego, CA, USA). Data were presented as mean ± SD. Continuous data normality was tested by the Shapiro–Wilk test. For continuous data with a normal distribution, an independent sample *t*-test was used for comparison between 2 groups, and one-way analysis of variance (ANOVA) or two-way ANOVA with Tukey HSD test was used for 3 or more groups. For continuous data that did not follow a normal distribution, the Mann–Whitney *U* test was used to analyze the difference between 2 groups, and the Kruskal–Wallis H test was used to analyze multiple groups of independent samples. The receiver operating characteristic (ROC) curve was conducted to evaluate the diagnostic performance of miR-330-3p. Statistical significance is achieved with a *P*-value below .05.

RESULTS

Baseline Demographic and Clinical Data Analysis Results

The baseline demographic and clinical data of all participants are shown in Table 1. The sex ($P = .575$), age ($P = .305$), body mass index (BMI) ($P = .062$), systolic blood pressure

Table 1. Baseline Demographic and Clinical Data

		CHD Patients		
Indicators	**Healthy Control**	**Simple CHD**	**CHD-PAH**	***P***
Sex (Female/Male)	30/20	38/22	61/28	.575
Age (years)	41.62 ± 0.57	41.73 ± 0.47	43.99 ± 0.44	.305
BMI (kg/m^2)	23.04 ± 2.53	22.68 ± 2.62	23.63 ± 2.27	.062
SBP (mm Hg)	119.2 ± 12.37	122.00 ± 15.29	124.50 ± 11.99	.070
DBP (mm Hg)	77.02 ± 10.01	78.57 ± 9.36	79.19 ± 9.33	.434
mPAP (mm Hg)	N/A	14.63 ± 2.85	48.39 ± 20.16	< .001
RAP (mm Hg)	N/A	6.77 ± 2.25	8.72 ± 1.93	< .001
PVR (Woods)	N/A	3.11 ± 0.35	7.34 ± 2.12	< .001
CI (L/min/m^2)	N/A	3.01 ± 0.60	2.93 ± 0.66	.507
BNP (pg/mL)	N/A	154.60 ± 83.46	286.40 ± 135.20	< .001

BMI, body mass index; BNP, B-type natriuretic peptide; CI, cardiac index; DBP, diastolic blood pressure; mPAP, mean pulmonary artery pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SBP, systolic blood pressure.

(SBP) (P = .070), and diastolic blood pressure (DBP) (P = .434) have no significant difference among healthy control, CHD, and CHD-PAH groups. Consistent with the diagnosis, patients with CHD-related PAH have higher mPAP (P < .001), right atrial pressure (RAP) (P < .001), pulmonary vascular resistance (PVR) (P < .001), and BNP levels (P < .001) than CHD patients. However, other right cardiac catheterization data, cardiac index (CI) (P = .507), have no significant difference.

MiR-330-3p Expression and Its Diagnostic Performance in CHD-Related PAH

The serum miR-330-3p expression levels in 3 groups of participants were measured by RT-qPCR. As displayed in Figure 1A, miR-330-3p levels were elevated in serum samples of CHD patients and CHD-PAH patients in contrast to healthy controls (n = 3, P < .001). Meanwhile, CHD-related PAH patients showed the highest miR-330-3p expression compared to the control group (n = 3, P < .001). The ROC curve indicated a high area under the ROC curve (AUC) in distinguishing CHD patients from healthy individuals (Figure 1B, AUC = 0.916) and differentiating CHD-related PAH patients from CHD patients (Figure 1C, AUC = 0.838).

Furthermore, the miR-330-3p levels were compared in mild PAH (M-CHD-PAH) and severe PAH (H-CHD-PAH) groups. Patients with severe PAH showed higher miR-330-3p levels (Figure 1D, n = 3, P < .001). The ROC curve showed that miR-330-3p had a relatively high AUC value in distinguishing severe patients from mild patients (Figure 1E, AUC = 0.810).

Silencing miR-330-3p Repressed Hypoxia-induced Viability, Migration, and Inflammation in HPASMCs

To observe the miR-330-3p expression during hypoxia in HPASMCs, the HPASMCs were exposed to hypoxia for 6 (P = .133), 12 (P = 0.002), 24 (P < .001), and 48 hours (P < .001). The RT-qPCR analysis indicated that miR-330-3p expression was increased after hypoxia treatment, especially at 24 h (Figure 2A, n = 3, P < .001). To establish a PAH model in vitro, HPASMCs were starved at DMEM medium without serum for 24 hours, and then cultured in an incubator under hypoxia conditions with a supply of 3% O_2, 5% CO_2, and balanced N_2 at 37°C for 24 hours. Hypoxia-induced miR-330-3p levels were upregulated in HPASMCs while a miR-330-3p inhibitor reversed the increased miR-330-3p levels (Figure 2B, n = 3, P < .001). The CCK-8 assay indicated that hypoxia facilitated cell viability while miR-330-3p knockdown diminished the increased cell viability caused by hypoxia (Figure 2C, n = 3, P < .001). Similarly, the migration assay revealed that hypoxia promoted cell migration while silencing miR-330-3p reversed the hypoxia-upregulated migration (Figure 2D, n = 5, P < .001). Moreover, downregulation of miR-330-3p inhibited hypoxia-induced high expression of inflammatory factors interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α in HPASMCs) (Figures 2E and F, n = 3, P < .001).

KLF10 Was a Putative Target of miR-330-3p

The binding sites between miR-330-3p and KLF10 were predicted from TargetScan and displayed in Figure 3A. The serum KLF10 levels decreased in patients with CHD or CHD-PAH compared with healthy controls (Figure 3B, n = 3,

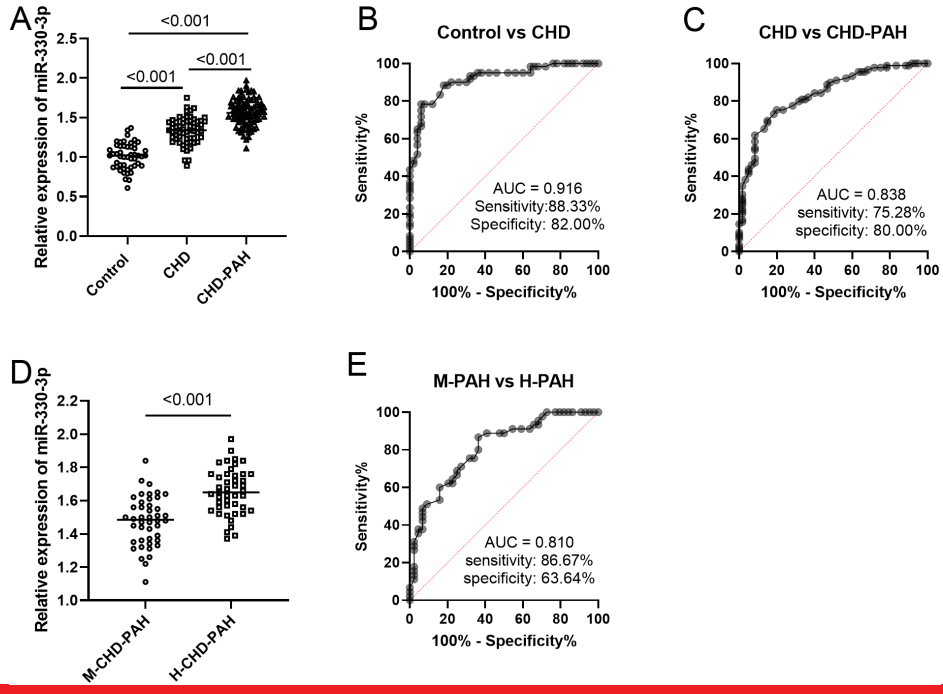

Figure 1. miR-330-3p expression and its diagnostic performance in patients with CHD-PAH. A. Serum miR-330-3p expression was elevated in CHD-PAH and CHD patients. B. The ROC curve indicated that miR-330-3p had a high AUC in distinguishing CHD patients from healthy individuals. C. miR-330-3p could differentiate CHD-PAH patients from CHD patients. D. miR-330-3p expression levels were higher in patients with severe PAH than in those with mild PAH. E. miR-330-3p expression had a relatively high diagnostic value in distinguishing patients with severe PAH from mild PAH.

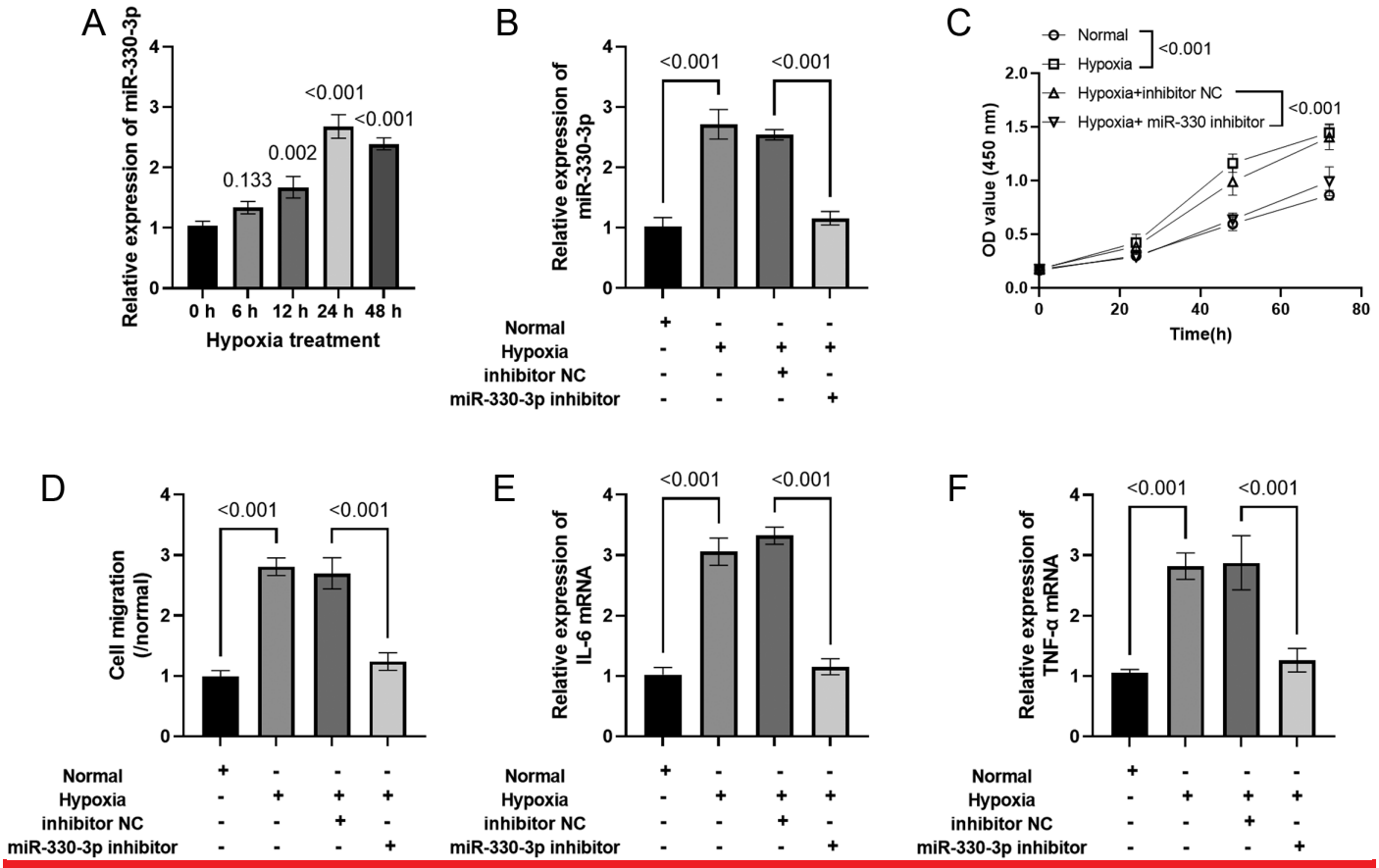

Figure 2. Silencing miR-330-3p repressed hypoxia-induced viability, migration, and inflammation in HPASMCs. A. miR-330-3p was upregulated in HPASMCs that were exposed to hypoxia at different times. B. Hypoxia treatment increased miR-330-3p expression, while miR-330-3p inhibitors decreased miR-330-3p expression. C. Cell viability was measured by CCK-8 assay. D. Transwell migration assay was used to measure the effects of miR-330-3p on cell migration. E and F. Hypoxia upregulated inflammatory factors IL-6 and TNF-α, while inhibition of miR-330-3p repressed hypoxia-upregulated IL-6 (E) and TNF-α (F) levels.

P < .001). Pearson correlation analysis revealed an inverse relationship between miR-330-3p and KLF10 levels in patients with CHD-related PAH (Figure 3C, *P* < .001). Dual-luciferase reporter assay indicated that luciferase activity was decreased in HPASMCs cotransfected with miR-330-3p mimic and KLF10-WT plasmids, while no changes were observed in cells cotransfected with KLF10-MUT and miR-330-3p mimic (Figure 3D, n = 3, *P* < .001). Furthermore, KLF10 mRNA levels were inhibited in miR-330-3p-elevated cells (n = 3, *P* = .003) but were increased in miR-330-3p-inhibited HPASMCs (n = 3, *P* = .010) (Figure 3E). The levels of KLF10 were also detected in HPASMCs exposed to hypoxia. Compared to the normoxia group, KLF10 mRNA levels were decreased after hypoxia treatment, especially at 24 hours (Figure 3F, n = 3, *P* < .001).

Interfering of KLF10 Partially Reversed the Influence of miR-330-3p on Hypoxia-Treated HPASMCs

Quantitative polymerase chain reaction analysis revealed that KLF10 expression was increased in miR-330-3p knockdown cells (n = 3, *P* = .003) while it was decreased by si-KLF10 in HPASMCs (n = 3, *P* < 0.001) (Figure 4A). Cell counting kit-8 assay revealed that silencing miR-330-3p increased cell viability, while the effect was reversed when transfected with si-KLF10 (Figure 4B, n = 3, *P* < .001). The same trends in migration abilities were observed in HPASMCs (Figure 4C, n = 5, *P* < .001). Furthermore, the downregulation of KLF10 reversed the decreased inflammatory factors induced by the downregulation of miR-330-3p in hypoxia-treated HPASMCs (Figures 4D and E, n = 3, *P* < .05).

DISCUSSION

As a common complication in patients with CHD, PAH tends to appear with more clinical symptoms and further deterioration of the condition. In the early reversible stage, CHD-PAH may be completely cured through timely intervention with blockage or closed surgery. In the late stage of irreversibility, shunt closed not only cannot reverse the rise in pulmonary artery pressure but is likely to cause disease progression and lead to a worse prognosis.[18] However, the early clinical symptoms of AH-CHD patients are non-specific, making the early diagnosis of the disease difficult. Right cardiac catheterization is the gold standard for the diagnosis of CHD-PAH, but it is invasive and limited in clinical application. The pathophysiological manifestations of PAH are closely related to the differential expression of various miRNAs, and miRNA detection is simple and has a low risk of damage,

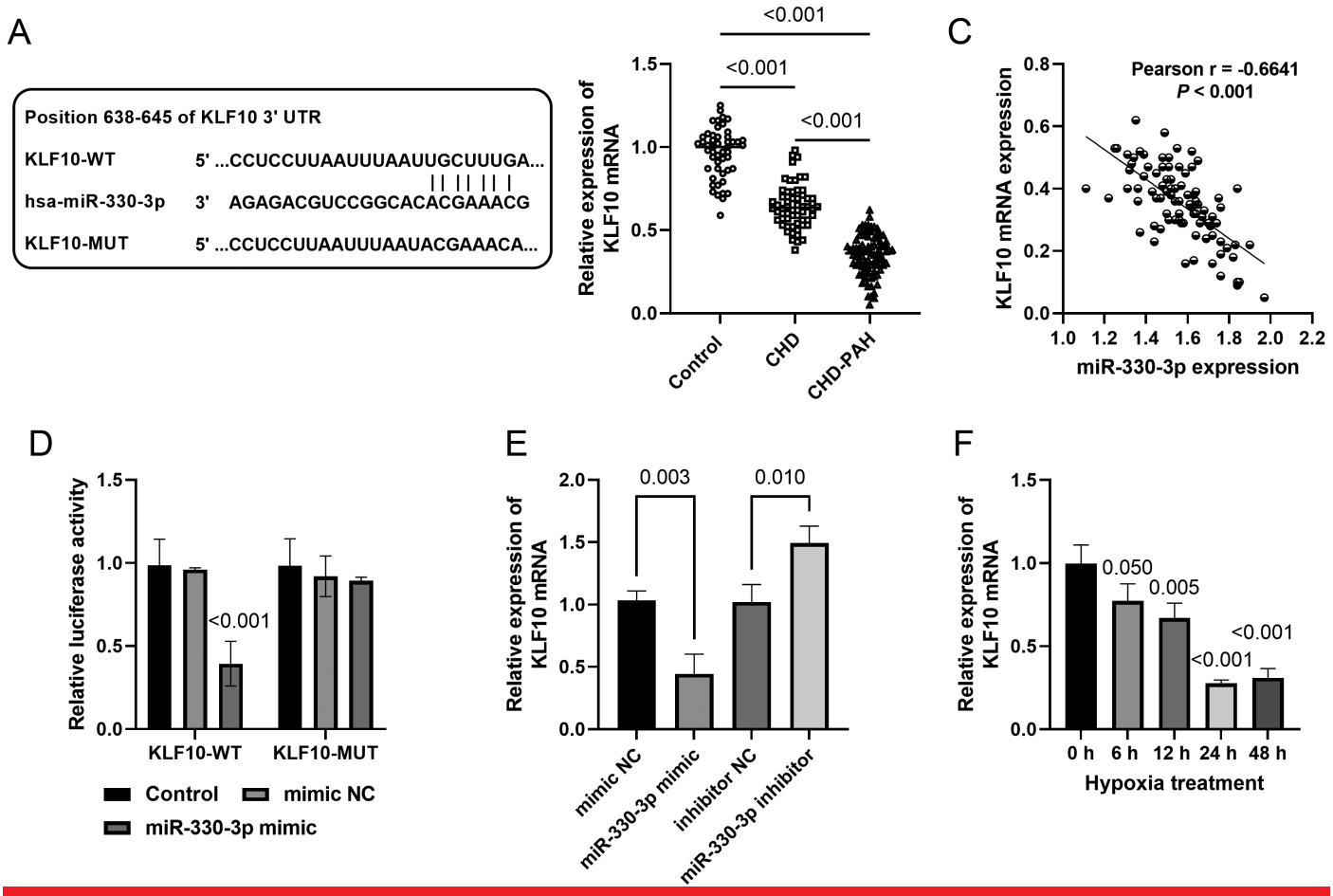

Figure 3. KLF10 was a direct target of miR-330-3p. A. TargetScan predicted the binding sites between miR-330-3p and KLF10. B. KLF10 mRNA levels were decreased in CHD and CHD-PAH patients, especially in CHD-PAH patients. C. Pearson correlation analysis indicated a negative correlation between miR-330-3p and KLF10 mRNA levels in CHD-PAH patients. D. Dual-luciferase reporter assay verified the targeting relationship between miR-330-3p and KLF10. E. KLF10 mRNA expression was decreased in miR-330-3p-upregulated HPASMCs, while it increased in miR-330-3p-inhibition HPASMCs. F. KLF10 mRNA levels were decreased in hypoxia-treated HPASMCs at different time points, especially at the 24-hour point.

which is conducive to large-scale screening, improves the efficiency of diagnosis and treatment, and is more practical and operable.[19]

The aberrant expression of miR-330-3p is involved in various diseases and plays crucial roles in cellular activities.[20,21] For instance, miR-330-3p acts as an oncogenic or suppressive factor in different tumors, such as breast cancer,[13] lung cancer,[22] and glioma.[22] A recent study indicated that plasma miR-330-3p decreased atrial fibrillation compared with paroxysmal supraventricular tachycardia, and it can be a cardiac-specific biomarker for atrial remodeling progression and atrial fibrillation patients.[15] These studies imply that abnormal expression of miR-330-3p may also be involved in heart-related disease. In the current study, 3 groups of participants were enrolled, including healthy controls, CHD patients, and CHD-PAH patients. These participants have no statistical difference in age, sex, BMI, SBP, and DBP, suggesting that these participants are comparable. The right cardiac catheterization indicators are consistent with the diagnosis of CHD-PAH patients. The miR-330-3p was upregulated in CHD patients and CHD-PAH patients compared to healthy individuals, especially in patients with CHD-PAH, which suggests that miR-330-3p may be involved in the progression of CHD to CHD-PAH.

Increasingly, studies demonstrated the diagnostic value of miRNAs in pulmonary arterial hypertension with congenital heart disease.[16,23] For instance, decreased circulating miR-509-3p has diagnostic significance in PAH with CHD.[24] MiR-27b was upregulated, and miR-451 expression was decreased in patients with CHD-PAH, both of which had diagnostic value and were risk factors for CHD-PAH.[10] In this study, serum miR-330-3p displayed diagnostic significance in distinguishing CHD-PAH patients from CHD patients and healthy individuals, suggesting that miR-330-3p might have diagnostic value in predicting patients with CHD-PAH. Moreover, serum miR-330-3p has potential value in differentiating CHD-PAH patients with different severities, indicating that miR-330-3p may be closely related to the severity of the disease.

Previous studies demonstrated that hypoxia exposure plays a crucial role in triggering the proliferation of HPASMCs,

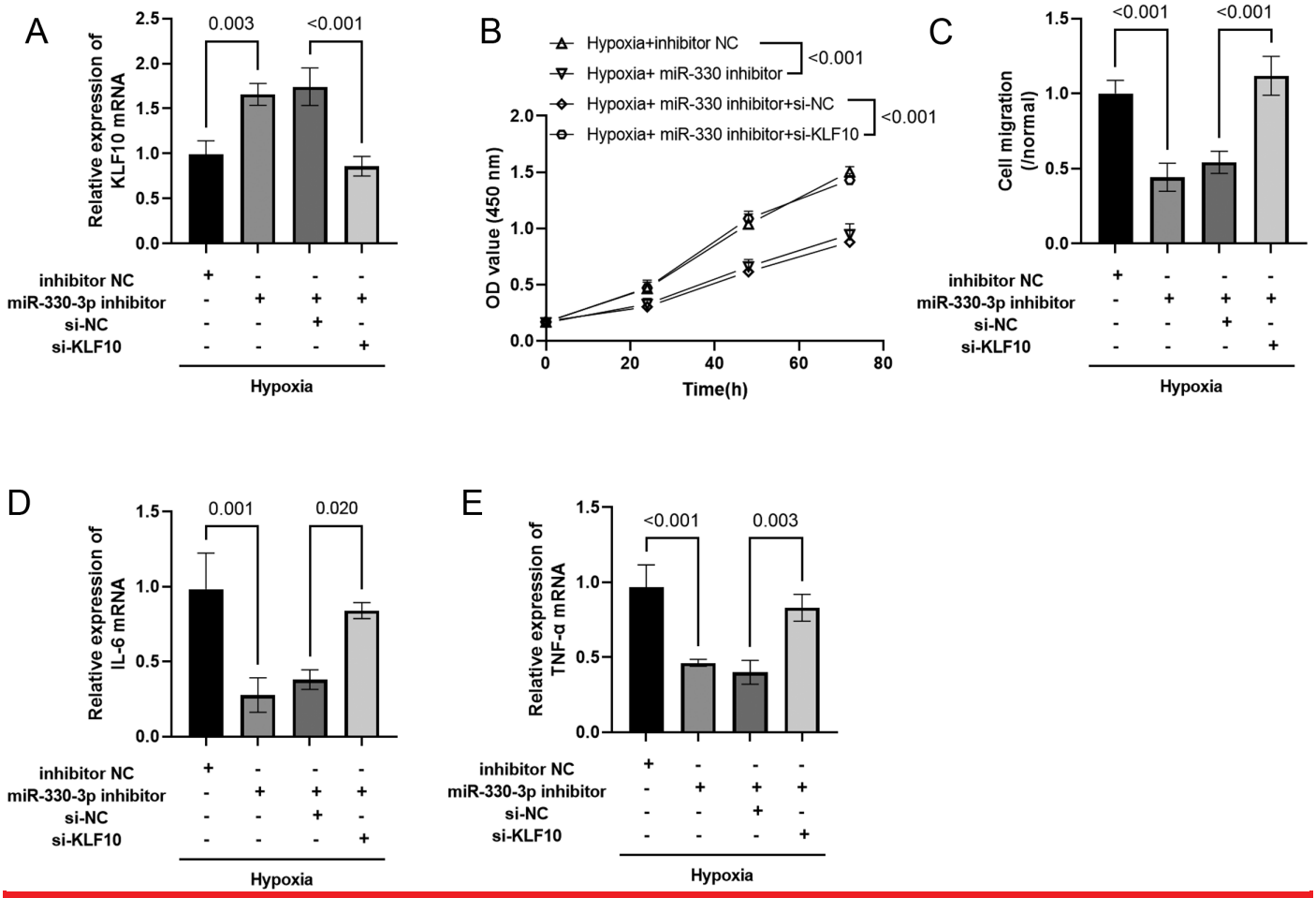

Figure 4. Interfering with KLF10 partially reversed the influence of miR-330-3p on hypoxia-treated HPASMCs. A. RT-qPCR detected the KLF10 levels after co-transfection in hypoxia-treated HPASMCs. B. Downregulation of KLF10 partially reversed the influence of miR-330-3p on viability in hypoxia-treated HPASMCs. C. The decreased cell migration by miR-330-3p inhibitor was reversed after si-KLF10 transfection. D and E. Downregulation of KLF10 reversed the decreased inflammatory factors induced by the downregulation of miR-330-3p in hypoxia-treated HPASMCs.

leading to subsequent vascular remodeling and the development of PAH.[25] Numerous studies reported that hypoxia-induced miRNAs participate in the progression of PAH. For instance, inhibition of miR-155-5p suppressed cell proliferation, migration, and cell cycle progression of hypoxia-stimulated PASMCs by regulating PYGL expression.[26] In this study, hypoxia observably promoted cell proliferation and migration of HPASMCs. Silencing miR-330-3p weakened the hypoxia-promoted viability and migration. Growing studies indicated that inflammatory factors were higher in CHD-PAH patients than in CHD and healthy controls.[27,28] Consistently, herein, inflammatory factors IL-6 and TNF-α were increased after hypoxia treatment. Importantly, the knockdown of miR-330-3p reduced the hypoxia-induced high IL-6 and TNF-α levels, which suggests that silencing miR-330-3p might have protective effects in CHD-PAH. Furthermore, KLF10 was a target of miR-330-3p. KLF10 was downregulated in CVB3 infection-induced cardiac in vitro acute myocarditis.[29] In this study, KLF10 expression was decreased in CHD-PAH compared with CHD patients and healthy individuals. Moreover, KLF10 deficiency partially reversed the protective effect of miR-330-3p knockdown on hypoxia-induced HPASMCs, which suggests that silencing of miR-330-3p may play a cardioprotective role in CHD-PAH by targeting KLF10.

Study Limitations

Several limitations should be acknowledged. First, the sample size of this study was relatively limited. Although the size was adequately powered to show the diagnostic value of miR-330-3p in patients with CHD-PAH, its clinical significance should be verified in a larger cohort of participants. Besides, since the regulatory mechanisms of miRNAs have not been fully clarified and they are involved in the expression of more than one gene, the clinical diagnostic value of a single miR-330-3p needs to be validated by additional research. Secondly, the functional role of miR-330-3p in CHD-PAH was investigated in vitro cellular experiments, which will be explored in vivo in future studies. Finally, the precise molecular mechanism behind the observed influence of miR-330-3p on CHD-PAH is not encompassed in this current research and needs to be explored in future investigations.

CONCLUSION

In conclusion, miR-330-3p is upregulated in the serum samples of patients with CHD-PAH and hypoxia-induced HPASMCs, suggesting that miR-330-3p may have putative value for early warning and screening of CHD-PAH. Knockdown of miR-330-3p can inhibit the proliferation, migration, and inflammation of HPASMCs by regulating KLF10, indicating that miR-330-3p is expected to be a novel small-molecule drug for the targeted treatment of CHD-PAH.

Artificial Intelligence (AI)-Assisted Technology: This manuscript does not use artificial intelligence (AI)-assisted technologies.

Ethics Committee Approval: The study was performed in line with the principles of the Declaration of Helsinki. All experiments are subject to consent by the patients and signed informed consent in accordance with the standards of Ethics Committee of Guizhou Provincial People's Hospital (Date: 23/6/2021/ No. 2021-015).

Informed Consent: Written informed consent was obtained from the patients who agreed to take part in the study.

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REFERENCES

1. Goldstein SA, Krasuski RA. Pulmonary hypertension in adults with congenital heart disease. *Cardiol Clin*. 2022;40(1):55-67. [CrossRef]
2. Ho L, Hossen N, Nguyen T, Vo A, Ahsan F. Epigenetic mechanisms as emerging therapeutic targets and microfluidic chips application in pulmonary arterial hypertension. *Biomedicines*. 2022;10(1):170. [CrossRef]
3. Naeije R, Richter MJ, Rubin LJ. The physiological basis of pulmonary arterial hypertension. *Eur Respir J*. 2022;59(6):2102334. [CrossRef]
4. Maron BA, Abman SH, Elliott CG, et al. Pulmonary arterial hypertension: diagnosis, treatment, and novel advances. *Am J Respir Crit Care Med*. 2021;203(12):1472-1487. [CrossRef]
5. Cao Y, Zheng M, Sewani MA, Wang J. The miR-17-92 cluster in cardiac health and disease. *Birth Defects Res*2024;116(1):e2273. [CrossRef]
6. Chiang MH, Liang CJ, Lin LC, et al. miR-26a attenuates cardiac apoptosis and fibrosis by targeting Ataxia-telangiectasia mutated in myocardial infarction. *J Cell Physiol*. 235(9):6085-6102. [CrossRef]
7. Liu X, Li H, Hastings MH, et al. miR-222 inhibits pathological cardiac hypertrophy and heart failure. *Cardiovasc Res*. 2024;120(3):262-272. [CrossRef]
8. Walasik I, Klicka K, Grzywa TM, et al. Circulating miR-3613-5p but not miR-125b-5p, miR-199a-3p, and miR-451a are biomarkers of endometriosis. *Reprod Biol*. 2023;23(4):100796. [CrossRef]
9. Xie P, Wang Z, Chen X, Han Y, Yang M, Ye Z. Regulations of LINC0196/miR-584-5p/miR-34a-5p/TRIM59 on progression of pediatric neuroblastoma. *Cell Mol Biol (Noisy-le-grand)*. 2022;68(6):117-123. [CrossRef].
10. Long L, Xiao Y, Yin X, Gao S, Zhou L, Liu H. Expression of serum miR-27b and miR-451 in patients with congenital heart disease associated pulmonary artery hypertension and risk factor analysis. *Exp Ther Med*. 2020;20(4):3196-3202. [CrossRef]
11. Chang WT, Wu CC, Lin YW, et al. Dynamic changes in miR-21 regulate right ventricular dysfunction in congenital heart disease-related pulmonary arterial hypertension. *Cells*. 2022;11(3):564. [CrossRef]
12. Zhang Q, Wang SF. miR-330 alleviates dextran sodium sulfate-induced ulcerative colitis through targeting IRAK1 in rats. *Kaohsiung J Med Sci*. 2021;37(6):497-504. [CrossRef]
13. Zhang Y, Guo C, Yang S, et al. NONHSAT021545/miR-330-3p/EREG: A cooperative axis in breast cancer prognosis and treatment. *J Clin Med*. 2023;12(7):2478. [CrossRef]
14. Shan E, Yu Y, Tang W, et al. miR-330-3p alleviates the progression of atherosclerosis by downregulating AQP9. *Funct Integr Genomics*. 2023;23(2):77. [CrossRef]
15. Harada M, Okuzaki D, Yamauchi A, et al. Circulating miR-20b-5p and miR-330-3p are novel biomarkers for progression of atrial fibrillation: intracardiac/extracardiac plasma sample analysis by small RNA sequencing. *PLoS One*. 2023;18(4):e0283942. [CrossRef]
16. Chang WT, Lee WC, Lin YW, et al. Transpulmonary expression of exosomal microRNAs in idiopathic and congenital heart disease-related pulmonary arterial hypertension. *J Am Heart Assoc*. 2023;12(23):e031435. [CrossRef]
17. Zhang Y, Peng B, Han Y. MiR-23a regulates the proliferation and migration of human pulmonary artery smooth muscle cells (HPASMCs) through targeting BMPR2/Smad1 signaling. *Biomed Pharmacother*. 2018;103:1279-1286. [CrossRef]
18. Hascoët S, Pontailler M, Le Pavec J, et al. Transplantation for pulmonary arterial hypertension with congenital heart disease: impact on outcomes of the current therapeutic approach including a high-priority allocation program. *Am J Transplant*. 2021;21(10):3388-3400. [CrossRef]
19. Siregar FM, Hartopo AB, Haryana SM. Recent progress in the roles of microRNAs in pulmonary arterial hypertension associated with congenital heart disease. *Narra J* 2024. :4(1): e579. [CrossRef]
20. Wu W, Liang D. Expression and related mechanisms of miR-330-3p and S100B in an animal model of cartilage injury. *J Int Med Res*. 2021;49(9). :3000605211039471. [CrossRef]
21. Zhao X, Chen GQ, Cao GM. Abnormal expression and mechanism of miR-330-3p/BTG1 axis in hepatocellular carcinoma. *Eur Rev Med Pharmacol Sci*. 2019;23(16):6888-6898. [CrossRef]
22. Shen L, Yi S, Huang L, et al. miR-330-3p promotes lung cancer cells invasion, migration, and metastasis by directly targeting hSOD2b. *Biotechnol Appl Biochem*. 2019;66(1):21-32. [CrossRef]
23. Chen W, Li S. Circulating microRNA as a novel biomarker for pulmonary arterial hypertension due to congenital heart disease. *Pediatr Cardiol*. 2017;38(1):86-94. [CrossRef]
24. Tang P. Clinical diagnostic value of circulating serum miR-509-3p in pulmonary arterial hypertension with congenital heart disease. *Hellenic J Cardiol HJC Hell Kardiol Epitheor*. 2020;61(1):26-30. [CrossRef]
25. Siques P, Pena E, Brito J, El Alam S. Oxidative stress, kinase activation, and inflammatory pathways involved in effects on smooth muscle cells during pulmonary artery hypertension

under hypobaric hypoxia exposure. *Front Physiol*. 2021;12:690341. [CrossRef]
26. Wang G, Tao X, Peng L. miR-155-5p regulates hypoxia-induced pulmonary artery smooth muscle cell function by targeting PYGL. *Bioengineered*. 2022;13(5):12985-12997. [CrossRef]
27. Low A, George S, Howard L, Bell N, Millar A, Tulloh RMR. Lung function, inflammation, and endothelin-1 in congenital heart disease-associated pulmonary arterial hypertension. *J Am Heart Assoc*2018;7(4):e007249. [CrossRef]
28. Hirsch K, Nolley S, Ralph DD, et al. Circulating markers of inflammation and angiogenesis and clinical outcomes across subtypes of pulmonary arterial hypertension. *J Heart Lung Transplant Off Publ Int Soc Heart Transplant*. 2023;42(2):173-182. [CrossRef]
29. Yang J, Zhang H, Wang X, et al. Kruppel-like factor 10 protects against acute viral myocarditis by negatively regulating cardiac MCP-1 expression. *Cell Mol Immunol*. 2021;18(9):2236-2248. [CrossRef]