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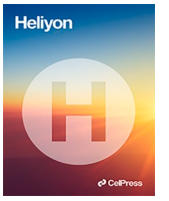

MiR-122-5p as a potential regulator of pulmonary vascular wall cell in idiopathic pulmonary arterial hypertension

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A R T I C L E I N F O

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A B S T R A C T

MicroRNAs (miRNAs) are versatile regulators of pulmonary arterial remodeling in idiopathic pulmonary arterial hypertension (IPAH). We herein aimed to characterize miRNAs in peripheral blood mononuclear cell (PBMC) and plasma exosomes, and investigate specific miRNA expression in pulmonary artery cells and lung tissues in IPAH. A co-dysregulated miRNA was identified from the miRNA expression profiles of PBMC and plasma exosomes in IPAH. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed the potential function of differentially expressed miRNAs. Real-time quantitative reverse transcription polymerase chain reaction was used to validate the expression of specific miRNAs in hypoxia-induced pulmonary microvascular endothelial cells (PMECs), pulmonary artery smooth muscle cells (PASMCs), pericyte cells (PCs), and lung tissues of patients with IPAH and rats. Finally, the miRNA-mRNA mechanisms of miR-122-5p were predicted. MiR-122-5p was the only co-upregulated miRNA in PBMC and plasma exosomes in patients with IPAH. Functional analysis of differentially expressed miRNAs revealed associations with the GO terms "transcription, DNA-templated," "cytoplasm," and "metal ion binding" in both PBMC and plasma exosomes, KEGG pathway MAPK signaling in PBMC, and KEGG-pathway human papillomavirus infection in plasma exosomes. Hypoxic PMECs and PCs, lung tissue of patients with IPAH, and rats showed increased expression of miR-122-5p, but hypoxic PASMCs showed decreased expression. And miR-122-5p mimics and inhibitor affected cell proliferation. Finally, miR-122-5p was found to potentially target DLAT (in lung tissue) and RIMS1 (in PMECs) in IPAH. According to the dual-luciferase assay, miR-122-5p bound to DLAT or RIMS1. In studies, DLAT imbalance was associated with cell proliferation and migration, RIMS1 is differentially expressed in cancer and correlated with cancer prognosis. Our

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findings suggest that the miR-122-5p is involved in various biological functions in the adjacent vascular wall cells in IPAH.

1. Introduction

The incidence and prevalence rates of pulmonary arterial hypertension (PAH) were reported to be 6 and 48–55 cases/million adults [1], respectively. PAH is primarily characterized by vascular remodeling resulting from endothelial dysfunction, excessive proliferation of pulmonary artery smooth muscle cells (PASMCs), and reduced pericyte coverage of pulmonary microvessels [2,3]. Right ventricular hypertrophy leads to right heart failure after pulmonary artery remodeling leads to increased pulmonary vascular resistance [4,5]. At present, targeted drug therapy for PAH mainly includes phosphodiesterase type 5 inhibitors, endothelin receptor antagonists, and prostacyclin analogues [6]. Idiopathic pulmonary arterial hypertension (IPAH) is the common subtype (accounting for 50–60 % of all cases) [1]. However, the etiology of IPAH remains unclear. The drugs currently used to treat IPAH are not have ideal effects on the disease. As a result, most patients have a poor prognosis, and do not show any improvement in their quality of life even after the treatment [7]. Therefore, the pathogenesis of IPAH needs to be explored, and several studies have shown that miRNAs contribute to its occurrence and development [8,9].

MiRNAs are endogenous approximately 22 nt RNAs [10], which mainly mediate the inhibition of translation and cutting and degradation of mRNA as well as play roles in cell growth and development, cell proliferation, apoptosis, organ formation, fat metabolism, DNA methylation, and mRNA transcription promotion [11–13]. MiRNAs are widely found in various tissues and body fluids in humans, among which miRNA expression in peripheral blood changes as the physiological and pathological conditions of individuals change [14]. Dysregulation of miRNAs can contribute to proliferative vasculopathy [15,16]. For example, a previous study showed that miR-124 modulated the activated phenotype of pulmonary vascular cells, which was reversed by HDAC inhibitors 16. These HDAC inhibitors also restored the levels of mature miR-124 [17]. Another study confirmed that miR-483 in endothelial cells inhibited inflammatory and fibrogenic response targets of PH-related genes, such as transforming growth factor-β, interleukin-1β, and endothelin-1 [18]. Moreover, the study found a new pathway of synergistic effect of miR-125a, BMPR2 and CDKN, and the inhibitor controlled the proliferation phenotype of endothelial cells [19]. High-throughput study of miRNA expression in patients with IPAH is lacking, so it is unclear how miRNAs contribute to IPAH development.

Given the significant role of miRNAs in IPAH, it is crucial to explore their regulatory functions. In the previous research, miR-122-5p was screened using a high-throughput sequencing strategy and found to be a co-upregulated miRNA in peripheral blood mononuclear cell (PBMC) and plasma exosomes in patients with IPAH. Additionally, the biological function of differentially expressed miRNAs was also investigated using bioinformatics analysis. The results of real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) revealed that miR-122-5p was upregulated in hypoxic pulmonary microvascular endothelial cells (PMECs), pericyte cells (PCs), and lung tissue of patients with IPAH and rats, but down-regulated in pulmonary artery smooth muscle cells (PASMCs). Finally, the miRNA-mRNA mechanism of miR-122-5p was predicted. Overall, our findings suggest that miR-122-5p regulates IPAH vascular wall cells.

2. Materials and methods

Ethical statement

ARRIVE guidelines were followed during all animal experiments, as well as UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63/EU. Animal experiments were approved by the Institutional Committee for Use and Care of Laboratory Animals of Tongji University (approval numbers: K20–150Y). Human experimentation was conducted in accordance with institutional and national ethical guidelines and the Helsinki Declaration (as revised in 2013). Prior to study initiation, the study protocol was reviewed and approved by Shanghai Pulmonary Hospital and Wuxi People's Hospital (approval numbers: K20–195Y and 2020-492). Prior to the commencement of the study, all participants willingly provided written informed consent and expressed their willingness to donate their tissue samples.

2.1. Sample selection

The patient group comprised six patients with IPAH admitted to Shanghai Pulmonary Hospital from May 2010 to April 2016 (three males and three females, age: >18 years). According to the guidelines of European Cardiology Society and European Respiratory Society, the diagnosis of IPAH was confirmed [20]. Patients were diagnosed by right heart catheterization (RHC) measured at rest, with mean pulmonary arterial pressure (mPAP) $\geq$ 25 mmHg and pulmonary arterial wedge pressure (PAWP) $\leq$15 mmHg. Patients with congenital heart disease, lung disease, connective tissue disease, portal hypertension, left heart disease, chronic thromboembolism, and portal hypertension were not included in the analysis. Individuals with acute or long-term illnesses that could impact the metabolism of hormones were also disqualified.

In the control group, six healthy physical examinees with matching age and gender were selected from the health examination center and patient group at the same time (three males and three females, age: >18). Inclusion criteria were as follows: (1) no previous history of other lung disease and related conditions, (2) no family history of related lung disease, (3) good health without drug and

alcohol dependence.

2.2. Extraction of PBMC and exosomes from plasma and miRNA extraction

Fresh blood was collected from the patient and control group and heparin lithium anticoagulant was added to the blood in a 4 ml tube. PBMC was isolated from whole blood using an extraction kit within 4 h of collection according to the manufacturer's instructions (Seebio, Shanghai, China). In addition, fresh blood samples were subjected to centrifugation at room temperature and 2500 g for a duration of 10 min. Subsequently, plasma was collected and transferred to a new tube, centrifuged at 15,000 g and 4 °C for 10 min to remove platelets. Exosomes were acquired after the final centrifugation step at 100,000 g for 2 h. Finally, exosomes were resuspended using 100 μL of PBS.

2.3. Determination of miRNAs

Oe Biotech Biotechnology Co. (Shanghai, China) carried out high-throughput sequencing. After converting the basic read to sequential data (known as raw data/reading), we obtained <15 nt and >41 nt in the raw data for clean reads. Small RNA sequencing procedures and analysis were also performed by Oe Biotech Biotechnology Co. The clean readings were aligned with short RNAs from the Rfam database (v2.2.28+) and GenBank database (http://www.ncbi.nlm.nih.gov/) using BLAST (v2.2.28+) in order to identify and eliminate noncoding RNAs. The miRBase (v21) database (http://www.mirbase. org/) was used to identify known miRNAs, and known miRNA expression patterns in various samples were examined.

2.4. Identification and functional enrichment analysis of differentially expressed miRNAs

The following criteria were employed to screen for differentially expressed miRNAs: p value < 0.05 and $|\log2 \text{ fold change}| > 1$. MiRanda and TargetScan were combined with statistical analysis to identify and predict the differentially expressed mRNAs targeted by the miRNAs. Functional enrichment analysis of differentially expressed miRNAs-mRNAs was performed using the DAVID online tools. The R package clusterProfiler was utilized for carrying out an enrichment analysis of mRNAs using two sources, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG).

2.5. Cell culture, transfection, and proliferation assay

The culture of PMECs, PASMCs, and PCs involved the use of endothelial cell medium, smooth muscle cell medium, and pericyte medium, respectively. These cells were incubated at 37 °C with 5 % CO_2. Similarly, PMECs, PASMCs, and PCs were exposed to hypoxia in anoxic incubators containing 5 % O_2 for 48 h.

To manipulate miR-122-5p expression levels, PMECs, PASMCs, and PCs were transfected with either a 20 μM miRNA mimic (miR-122-5p) or a 20 μM miRNA inhibitor (anti-miR-122-5p) (GenePharma, Shanghai, China). This was carried out using Lipo2000 Transfection Reagent. To control for any nonspecific effects, control groups were included in which cells were treated with equivalent concentrations of nontargeting mimic or inhibitor negative control sequences.

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) from Dojindo in Japan. Cells were seeded into a 96-well plate, with a density of 3×10^3 cells per well. After 24 h of treatment with the miR-122-5p mimic or inhibitor under hypoxic conditions, the cells were incubated with 10 μL of CCK-8 solution at 37 °C for 60 min. The absorbance at 450 nm was then measured, and the relative proliferation level was normalized based on five independent experiments.

2.6. Animal models

All animal experiments were approved by the Committee of Tongji University School of Medicine for Animal Care and Use. Sprague-Dawley rats (n = 6) were injected subcutaneously with 25 mg/kg SU5416 dissolved in carboxymethylcellulose. Rats were then kept under a 10 % O_2 condition for 4 weeks, after which they were housed under normoxic conditions for 6 weeks. Monocrotaline-treated (MCT) Sprague-Dawley rats (n = 6) were subcutaneously injected with MCT (60 mg/kg) dissolved in sterile saline, followed by exposure to normoxic conditions for 4 weeks. Control animals in SuHx (n = 6) and MCT (n = 6) experiments received a single injection of carboxymethylcellulose and normal saline, respectively. Finally, rats were euthanized, their lung tissues were extracted and kept at −80 °C. Fresh lung tissue was fixed with 4 % paraformaldehyde and then paraffin-fixed lung tissue sections were stained with hematoxylin and eosin (H&E) staining (Solarbio, Beijing, China). Finally, these slices were photographed under an optical microscope (DP73, Olympus Corporation, Tokyo, Japan).

2.7. RNA isolation and RT-qPCR

Tissue samples from patients with IPAH who underwent lung transplants, as well as healthy donors, were collected and stored at −80 °C until being utilized. MiRNA was extracted from various sources such as lung tissue from humans and rats, PMECs, PASMCs, and PCs, using the miRNeasy Mini Kit (Qiagen, Hilden, Germany). The purity of the RNA was measured using a NanoDrop 2000 spectrophotometer at 260/280 nm, with a ratio ranging from 1.9 to 2.1. The levels of miR-122-5p were measured in PMECs, PASMCs, PCs, and lung tissue from both rats and humans using a SYBR Green-based RT-qPCR kit (TOYOBO, QPK201, Osaka, Japan). The specific

primer pairs used for the analysis are listed in Table 2.

2.8. Verifying the differentially expressed mRNA in IPAH samples using the GEO database

The mRNA microarray profiles GSE117261 and GSE15197 were obtained from the Gene Expression Synthesis (GEO) public database for analysis. Samples from both public databases included lung tissue. After downloading the microarray data, the online network tool GEO2R was used for analysis. The following criteria were used to define differentially expressed mRNAs in microarray: p value < 0.05 and $|\log2 \text{ fold change}| > 1$. The miRNA expression profiles in hypoxic PMECs used here were obtained from the results of our previous research. MiRNA was extracted using the same method and tested by the same method by the same company. The ClueGO algorithm was utilized on Cytoscape software in order to visualize and conduct biological analyses on the shared genes miR-122-5p found in both the Targentscan and miRWalk databases. This provided insight into the potential role of these genes.

2.9. Dual-luciferase activity reporter system

The dual-luciferase reporter assay was performed to verify that DLAT or RIMS1 was a target for miR-122-5p. Wild type and mutant sequences of DLAT or RIMS1 were inserted after the firefly luciferase gene in the pGL3 vector from Promega, USA. Lipofectamine 2000 was used to co-transfect miR-122-5p mimics and luciferase-reporter plasmids, following the manufacturer's instructions. MiR-122-5p mimics or a negative control were co-transfected with pGL3-DLAT and pGL3-RIMS1 vectors into 293T cells. After 48 h, firefly luciferase activity was measured using Promega's dual-luciferase assay kit and normalized to Renilla luciferase activity. Table 3 contained the primer and oligonucleotide sequences.

2.10. Statistical tools and methods

Identification of differentially expressed miRNAs between IPAH and healthy control tissue samples by t-test and limma package in R. Differentially expressed mRNAs in the microarray were defined based on the following criteria: p value < 0.05 and $|\log2 \text{ fold change}| > 1$. The volcano plots were generated utilizing bioinformatics software (bioinformatics.com), while other computational procedures involved the utilization of in-house programs coded in R. In terms of real-time PCR analysis, the fold change of miR-122-5p expression level was presented using the $2^{-\triangle\triangle Ct}$ method, and a p value below 0.05 was deemed statistically significant.

3. Results

3.1. Analysis of differentially expressed miRNAs

The research flow is shown in Fig. 1. We identified 62 and 13 miRNAs that were upregulated and downregulated, respectively, in the PBMC of patients with IPAH. The sequencing data quality was shown in Table S2 and Fig. S1. The volcano plot was produced that indicated the variation in miRNA expression with statistical significance between the two groups (Fig. 2A). Furthermore, an unsupervised hierarchical clustering analysis was conducted to generate a heatmap, depicting the differential expression of various miRNAs

Table 1
Top 20 upregulated and downregulated miRNAs in PBMC and plasma exosomes.

miRNA-diff-TOP20							
pulmonary artery smooth muscle cell (PBMC)				plasma exosomes			
miRNA-id	log2FC	p value	regulation	miRNA-id	log2FC	p value	regulation
miR-577	3.43373999	3.58E-3	up	miR-4443	4.44903123	8.03E-5	up
miR-541-3p	3.20415944	2.94E-2	up	miR-296-3p	4.13046122	1.98E-2	up
miR-5701	3.17879889	4.68E-2	up	miR-342-3p	4.10322342	6.35E-3	up
miR-5585-3p	2.68176650	1.71E-2	up	miR-122-5p	4.04185869	1.13E-4	up
miR-3653-3p	2.34198182	3.29E-3	up	miR-582-3p	3.96646148	1.17E-2	up
miR-3180-5p	1.92566120	4.45E-2	up	miR-1180-3p	3.80529681	1.04E-2	up
miR-210-5p	1.80443339	3.20E-2	up	miR-589-5p	3.14847621	6.86E-3	up
miR-193a-3p	1.79355866	2.06E-2	up	miR-1228-5p	2.87633413	9.73E-4	up
miR-6818-3p	1.78641333	3.17E-2	up	miR-150-5p	2.48557166	2.61E-4	up
miR-122-5p	1.51893884	4.70E-2	up	miR-15b-5p	2.36844246	2.62E-2	up
miR-4632-3p	1.45792633	3.69E-2	up	miR-139-5p	−1.24574676	7.44E-3	down
miR-6511b-3p	1.27729676	3.55E-2	up	miR-98-5p	−1.66091922	4.85E-2	down
miR-1908-5p	1.27536388	3.51E-2	up	miR-5189-5p	−2.87419833	1.12E-2	down
miR-204-5p	1.24283923	4.10E-2	up	miR-6859-5p	−4.99060794	1.15E-2	down
miR-1-3p	−1.39746196	3.72E-2	down	miR-195-5p	−5.45706641	2.87E-3	down
miR-1262	−1.85891954	4.02E-2	down	miR-149-5p	−5.51870890	3.75E-3	down
miR-4306	−1.92691549	2.04E-2	down	miR-6868-3p	−5.85217313	3.20E-2	down
miR-548ag	−2.20880029	1.21E-2	down	miR-3131	−6.02239589	3.93E-3	down
miR-133b	−2.31786715	1.92E-2	down	miR-411-3p	−6.70975095	2.78E-3	down
miR-4482-3p	−3.95419616	4.60E-2	down	miR-10a-3p	−7.54180993	9.79E-4	down

Table 2
Primers for miR-122-5p used in RT-qPCR.

miRNAs	Orientation	Sequences
has-miR-122-5p	Forward	ACACTCCAGCTGGGTGGAGTGTGACAATGG
	Reverse	GTGCAGGGTCCGAGGT
rno-miR-122-5p	Forward	ACACTCCAGCTGGGGTTTGTGGTAACAGTG
	Reverse	CAGTGCGTGTCGTGGAGT
U6	Forward	CTCGCTTCGGCAGCACA

Table 3
Primers and mimics in study.

Mimics and inhibitor sequences	
miR-27a-3p mimics	Sense (5′ to 3′) UGGAGUGUGACAAUGGUGUUUG
	Antisense (5′ to 3′) AACACCAUUGUCACACUCCAUU
Primers for dual luciferase activity reporter system	
DLAT-WT	F: ATCGCCGTGTAATTCTAGACATATGTGAATCTGACCAGTGCTTCCT
	R: GGCCGCCCCGACTCTAGAGAATTCCAAGGGAACAAVAGAACCACA
DLAT-MUT	F: TATTACATGGTACACAAGTGACCCCCCATATATTCCACACAGACTTTTACC
	R: GGTAAAAGTCTGTGTGGAATATATGGGGGGTCACTTGTGTACCATGTAATA
RIMS1- WT	F: ATCGCCGTGTAATTCTAGACATATGTGGCCTTGAATTTCCCTTGTG
	R: GGCCGCCCCGACTCTAGAGAATTCATGCAACATACAAGCTGGCC
RIMSI-MUT	F: GTTCTCCCTCCTTTTATAGATCCCCCCACTTTTGTGATTACACAAATAG
	R: CTATTTGTGTAATCACAAAAGTGGGGGGACTCATAAAAGGAGGGAGAAC

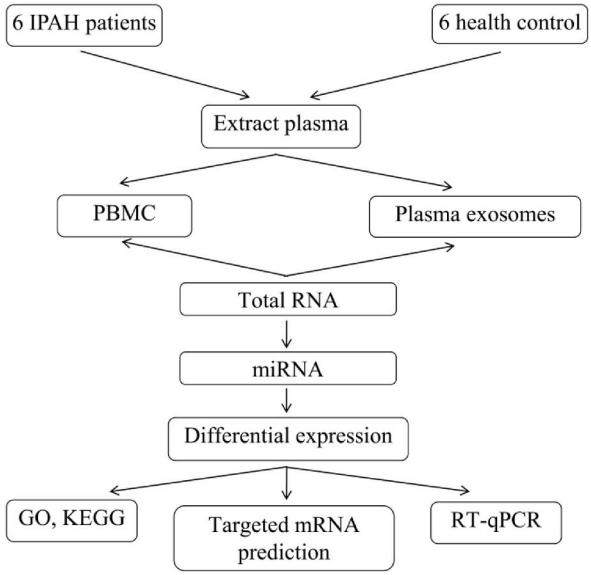

Fig. 1. The research flow chart.

across different groups (Fig. 2C). In addition, 59 and 21 miRNAs were upregulated and downregulated, respectively, in plasma exosomes of patients with IPAH. The volcano plot (Fig. 2B) and heatmap (Fig. 2D) were also produced on the basis of the differentially expressed miRNAs in plasma exosomes.

Table 1 presents the top 20 miRNAs that were identified as both upregulated and downregulated. Based on the data shown in Table 1, we identified that miR-122-5p as the only overlapping miRNA in the top 20 dysregulated miRNAs in PBMC and plasma exosomes in IPAH samples. Thus, Therefore, miR-122-5p may potentially have a significant impact on the progression of IPAH.

3.2. GO and KEGG analyses

20031 and 22472 genes were collected in PBMC and plasma exosomes respectively for enrichment analysis. As shown in Fig. 3, GO enrichment of differentially expressed miRNAs in PBMC and plasma exosomes was similar. Of the "Biological Process" GO terms, "transcription, DNA-templated" and "regulation of transcription, DNA-templated" were significant. Of the "Cellular Component" GO terms, "cytoplasm" and "nucleus" were significant. Of the "Molecular Function" GO terms, "metal ion binding" and "protein binding"

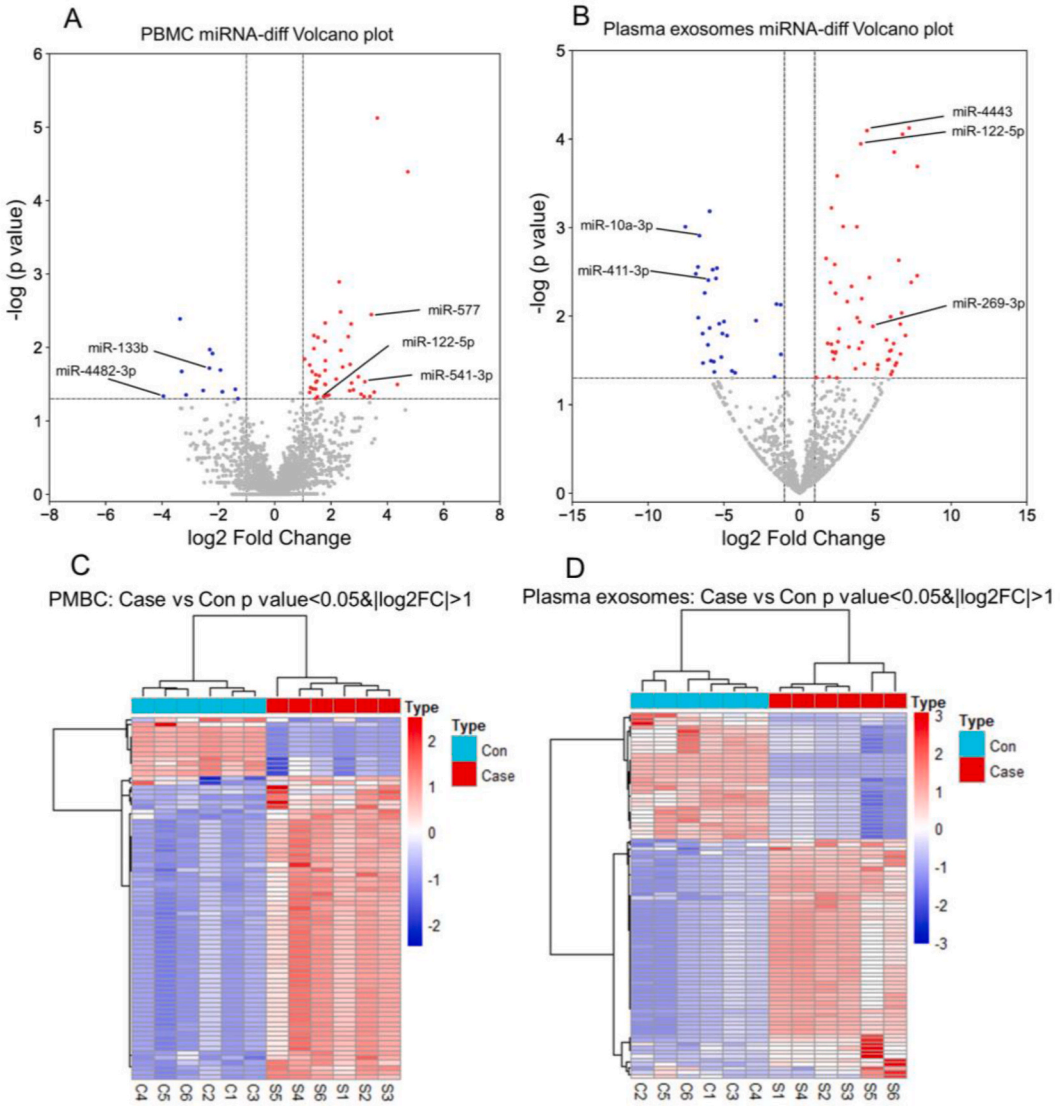

Fig. 2. Volcano plot of miRNA expression in PBMC (A) and plasma exosomes (B) and unsupervised hierarchical clustering analysis of differentially expressed miRNAs in PBMC (C) and plasma exosomes (D).

were significant. In the case of PBMC, KEGG pathway analysis demonstrated that the enriched miRNAs were associated with various pathways, including the MAPK signaling pathway, endocytosis, focal adhesion, and cancer pathways (Fig. 4A). Whereas, for plasma exosomes, KEGG pathway analysis indicated that miRNAs were enriched in human papillomavirus infection, calcium signaling pathway, Cushing syndrome, and oxytocin signaling pathway (Fig. 4B).

3.3. Verification of miR-122-5p expression

RT-qPCR was used to verify the expression of miR-122-5p in hypoxic PMECs, PASMCs, PCs, and lung tissues of patients with IPAH and rats. The pulmonary vascular wall of rats modeled by MCT (Fig. 5A) and SuHx combined with hypoxia modeling (Fig. 5B) was thickened and occluded, which showed that the animal model was successful. As illustrated in Fig. 5C–H, the expression of miR-122-5p was notably elevated in hypoxic PMECs and PCs, lung tissue of SuHx and MCT rats, as well as in lung tissue obtained from patients diagnosed with IPAH, when compared to control samples. In contrast, miR-122-5p was significantly downregulated in hypoxic PASMCs compared to the control group. Furthermore, miR-122-5p mimics and inhibitor can change the proliferation ability of PMECs, PASMCs and PCs (Fig. 5I–K) under hypoxia. MiR-122-5p mimics promoted the proliferation of PMECs and inhibited the proliferation of PASMCs. MiR-122-5p inhibitor inhibited the proliferation of PMECs and PCs, and promoted the proliferation of PASMCs.

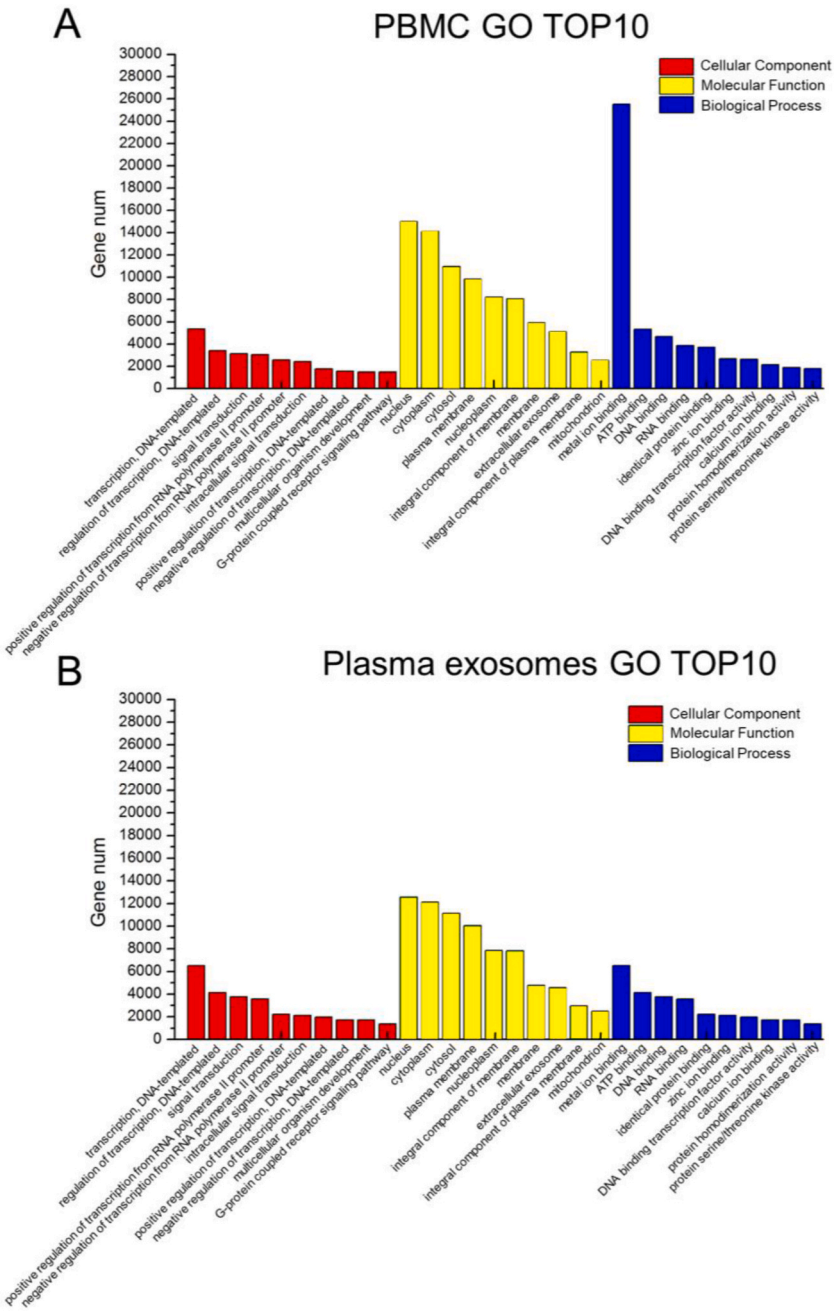

Fig. 3. The top 30 enriched GO terms related to the different miRNAs were mainly in biological processes, cellular components, and molecular function of miRNA in PBMC (A) and plasma exosomes (B).

3.4. Target prediction and functional analysis for the significant differentially expressed miRNAs

A total of 219 mRNAs and 34802 mRNAs of miR-122-5p were predicted using TargetScan and miRWalk, respectively. In addition, targeted mRNAs were verified as expressed downregulated mRNAs in public databases (GSE117261 and GSE15197) and DLAT was identified as intersection mRNA (Fig. 6B). The Targentscan and miRWalk databases were utilized by ClueGO to investigate the potential role of the shared genes associated with miR-122-5p. Furthermore, biological analyses were conducted on these shared genes. The first significantly enriched Gene Ontology (GO) term for Biological Processes (BP) was identified as "regulation of hydrogen peroxide-induced cell death," which accounted for 50 % of the total GO terms (Fig. 6A). Fig. 6C showed the differential expression of DLAT in the IPAH of GSE117261. The Targentscan and miRWalk databases overlapped with the predicted differentially expressed genes in PMECs and RIMS1 was found to be co-downregulated. The expression level of RIMS1 in PMECs is shown in Fig. 6D. To assess

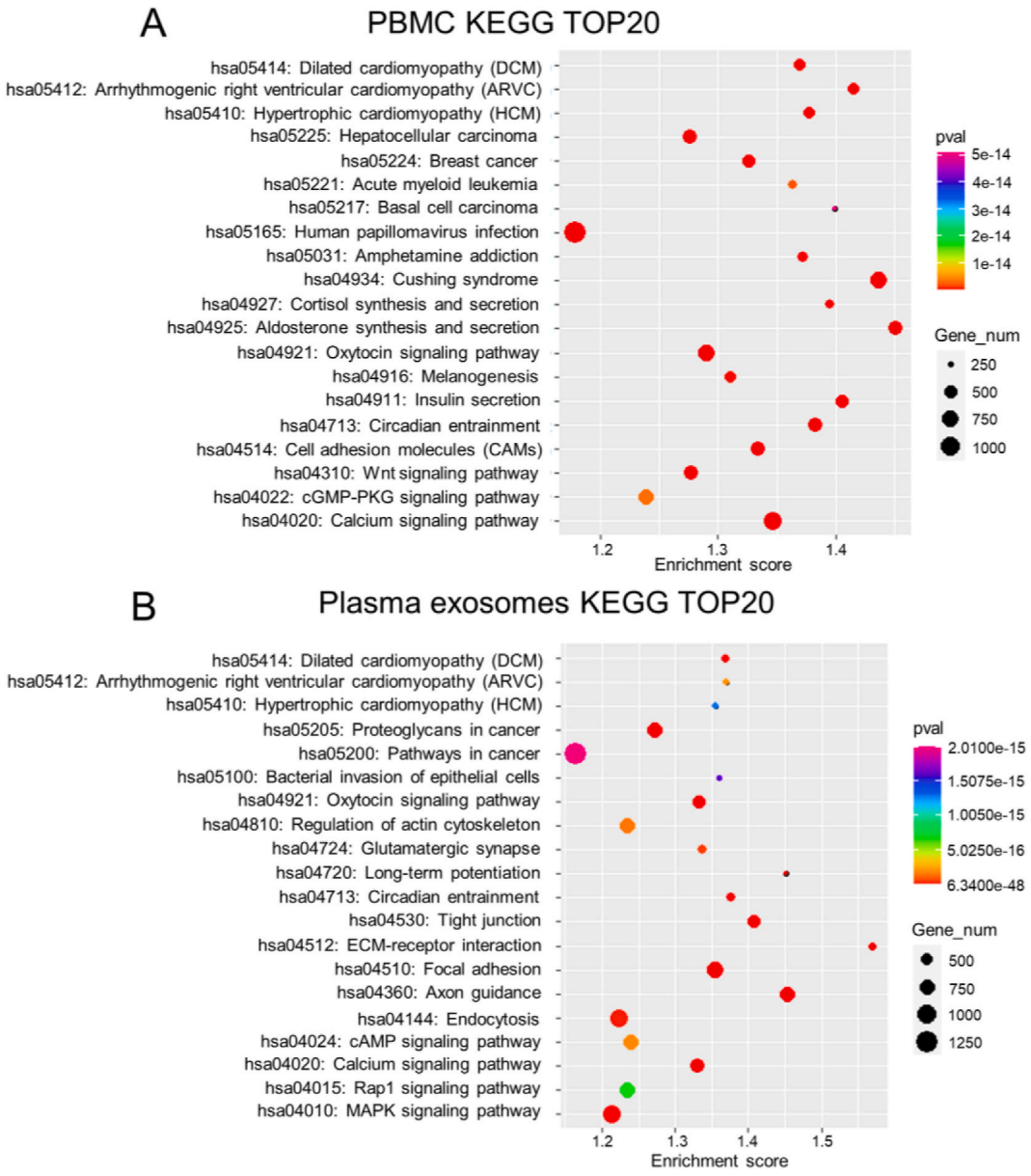

Fig. 4. KEGG pathway analysis of enriched miRNA-related pathways in PBMC (A) and plasma exosomes (B).

the interaction between miR-122-5p and its target genes DLAT or RIMS1, a dual-luciferase reporter assay was performed using a recombinant reporter plasmid containing the luciferase gene and either the DLAT or RIMS1 sequence (pGL3-DLAT or pGL3-RIMS1). Schematic recognition sites were shown in Fig. 6E and F. Co-transfection of pGL3-DLAT or pGL3-RIMS1 with miR-122-5p resulted in a significant reduction in firefly luciferase reporter activity (Fig. 6E and F), indicating that miR-122-5p can bind to DLAT or RIMS1.

Fig. 7 depicts the methodological procedures employed in this study, as well as the proposed mechanism of action of miR-122-5p in pulmonary artery cells and lung tissue.

4. Discussion

Several studies have used transcriptomics analysis for the identification of different miRNAs and mRNAs in serum and lung tissues from patients with IPAH [9,21]. However, studies related to miRNAs in IPAH are lacking. Our study indicated that miR-122-5p was upregulated in both PBMC and plasma exosomes in IPAH, and this result was verified in multiple lung cells and lung tissues.

MiRNA miR-122-5p is thought to be primarily involved in cell proliferation, apoptosis, and migration [22,23]. In addition, its association with the occurrence and progression of lung diseases has gradually come to light. For example, Wu et al. [24] discovered

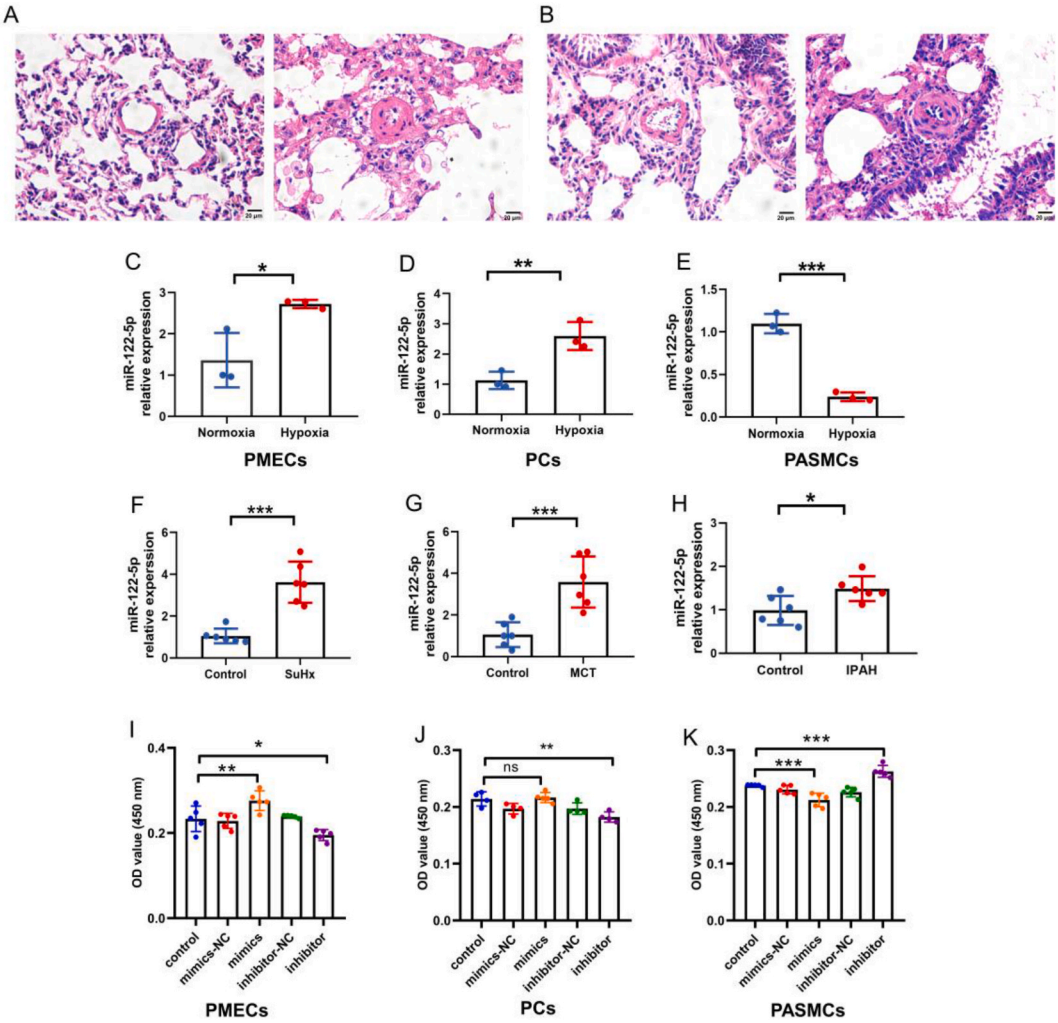

Fig. 5. Pulmonary artery of MCT modeling (A) and SuHx modeling (B) were performed using H&E, Scale bar: 20 μm. qPCR results for the expression of miR-122-5p in hypoxic and normoxic PMECs (C), PCs (D), and PASMCs (E). Expression of miR-122-5p in the lung tissue of MCT (F) and SuHx (G) rats compared with expression in the control and miR-122-5p expression in the lung tissue of controls and patients with IPAH (H). Effect of miR-122-5p mimics and inhibitor on proliferation ability of PMECs (I), PCs (J) and PASMCs (K) (*P < 0.05 , **P < 0.01 , ***P < 0.001).

that miR-122-5p plays a role in promoting the development of non-small cell lung cancer by downregulating p53 and activating the PI3K-Akt pathway. Research demonstrated that miR-122-5p induces exaggerated lung injury, inflammation, and oxidative stress in mice [25]. MiR-122-5p was found to regulate the promoter activity of DUSP4, which in turn negatively modulates ERK1/2 signaling to provide protection against acute lung injury [25]. Similarly, the role played by miR-122-5p in the pathogenesis and development of PH has been recognized. Indeed, miR-122-5p holds significance as a biomarker for hypertension and plays a crucial role in the diagnostic performance of PH. Dysregulation of miR-122-5p can serve as an indicator of PH risk [26]. Ma et al. [27] found that miR-122-5p was involved in the pathogenesis of pulmonary arterial hypertension (PAH) by analyzing the gene expression profile of PAH, and another study confirmed the miRNA expression is dysregulated in PAH, including the upregulation of miR-122-5p [28].

Whether in PBMC or plasma exosomes, the most significantly enriched GO terms were “transcription, DNA-templated”, “nucleus”, and “metal ion binding”. The bone morphogenetic proteins family has emerged in enriched genes of “transcription, DNA-templated”. BMPs and their receptors have been found to be essential for the development of PAH-induced right ventricular hypertrophy. Emerging data suggest that the restoration of BMP type II receptor (BMPR2) signaling in PAH could be a promising approach to prevent and reverse pulmonary vascular remodeling [29]. BMPR2 mutations have been identified in >70 % of familial and roughly 15 % of sporadic PAH cases [29]. SMAD9 was enriched in the pathway of “nucleus”, which mutation has been confirmed in PH. SMAD9 expression was increased by the activation of the BMP signaling, and SMAD9 reduced the BMP activity [30]. KEGG analysis showed that significantly enriched pathways in plasma exosomes included those related to cellular processes, such as calcium signaling pathway. This is similar to the enrichment of "metal ion binding" in GO analysis. Studies have shown that an increase in cytosolic Ca^{2+} concentration in PASMCs triggers pulmonary vasoconstriction and stimulates PASMC proliferation, leading to vascular wall thickening

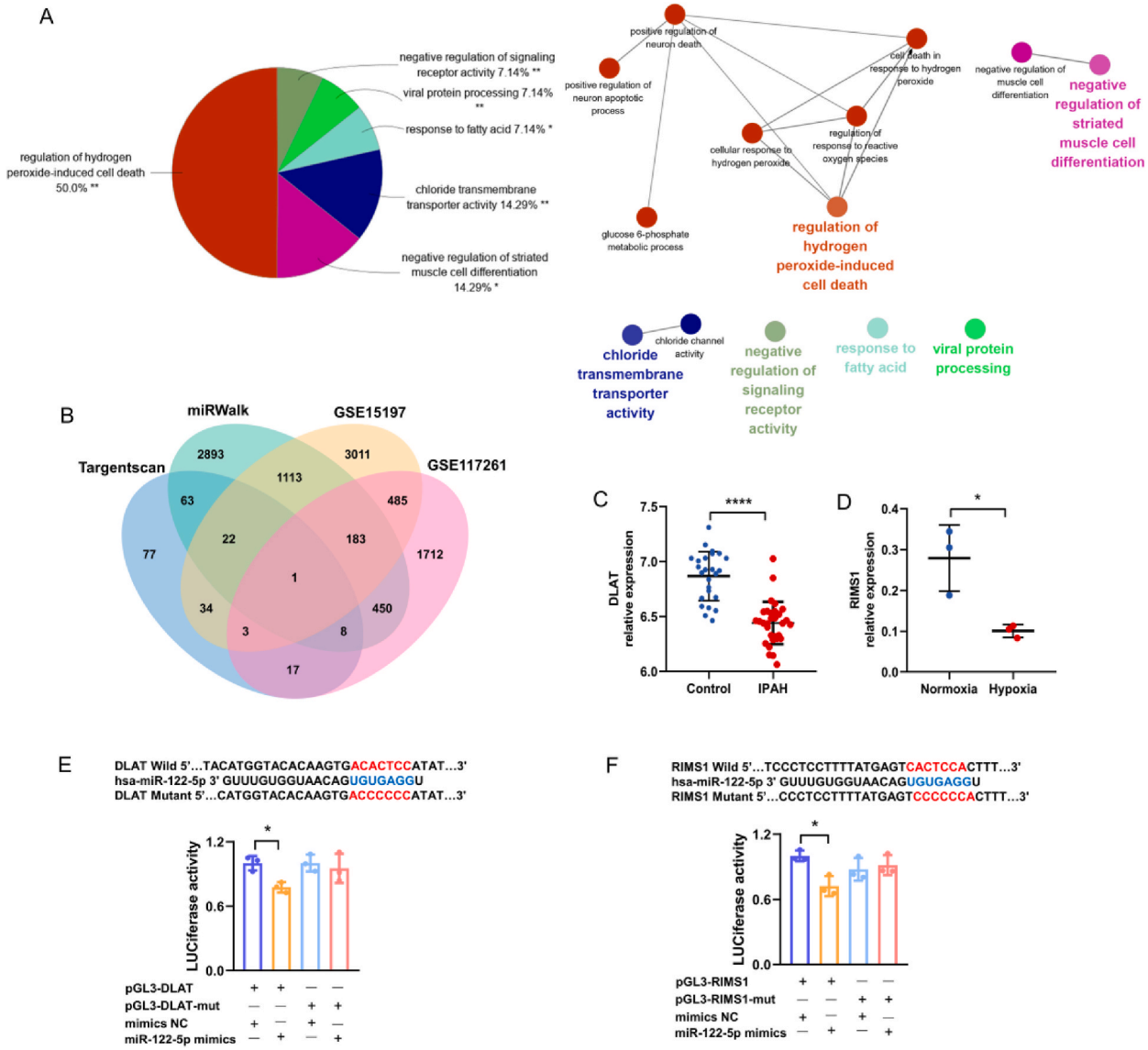

Fig. 6. (A) GlueGO analysis results of shared genes of miR-122-5p in Targentscan and miRWalk databases, the maximum enrichment pathway was regulation of hydrogen peroxide -induced cell death. (B) Venn diagram showing the overlap of the mRNAs of miR-122-5p according to TargetScan, miRWalk, and the datasets GSE116271 and GSE15197. (C) Expression of DLAT in the control and IPAH in dataset GSE116271. (D) Expression of RIMS1 in normoxic and hypoxic PMECs. Dual-luciferase assays were used to validate the interactions between DLAT (E) or RIMS1 (F) and miR-122-5p. (*P < 0.05 , **P < 0.01 , ***P < 0.001).

[31,32]. Besides, docosahexaenoic acid inhibits Ca^{2+} influx and downregulates calcium-sensing receptor by upregulating miR-16 in PASMCs [33]. These evidences show that "metal ion binding" is related to the pathogenesis of PH, especially Ca^{2+}. the KEGG analysis revealed that the MAPK signaling pathway exhibited the highest enrichment among peripheral blood mononuclear cells (PBMC). KEGG analysis also showed that the MAPK signaling pathway exhibited the highest enrichment among PBMC. This pathway is associated with cell proliferation, migration, differentiation, senescence, and apoptosis [34]. In addition, inhibition of the MAPK signaling pathway could alleviate hypoxia induced PH [35].

Compared with the controls, the expression of miR-122-5p was upregulated in hypoxia-induced PMECs and PCs and lung tissue of SuHx rats, MCT rats and patients with IPAH, whereas miR-122-5p expression was downregulated in hypoxia-induced PASMCs. Thus, miR-122-5p may be involved in different biological functions in the adjacent vascular wall cells of IPAH. To the best of our knowledge, relevant studies on PASMCs have not been previously published. However, a previous study reported that the role of miR-21 in pulmonary artery endothelial cells and PASMCs in PH differed [36]. Contrary to pulmonary artery endothelial cells, upregulation of miR-21 in PASMCs appears to be harmful [36]. Furthermore, cell proliferation experiments confirmed this result, and miR-122-5p mimics and inhibitor can change the proliferation ability of PMECs, PASMCs and PCs. Although the effect of miR-122-5p on PCs

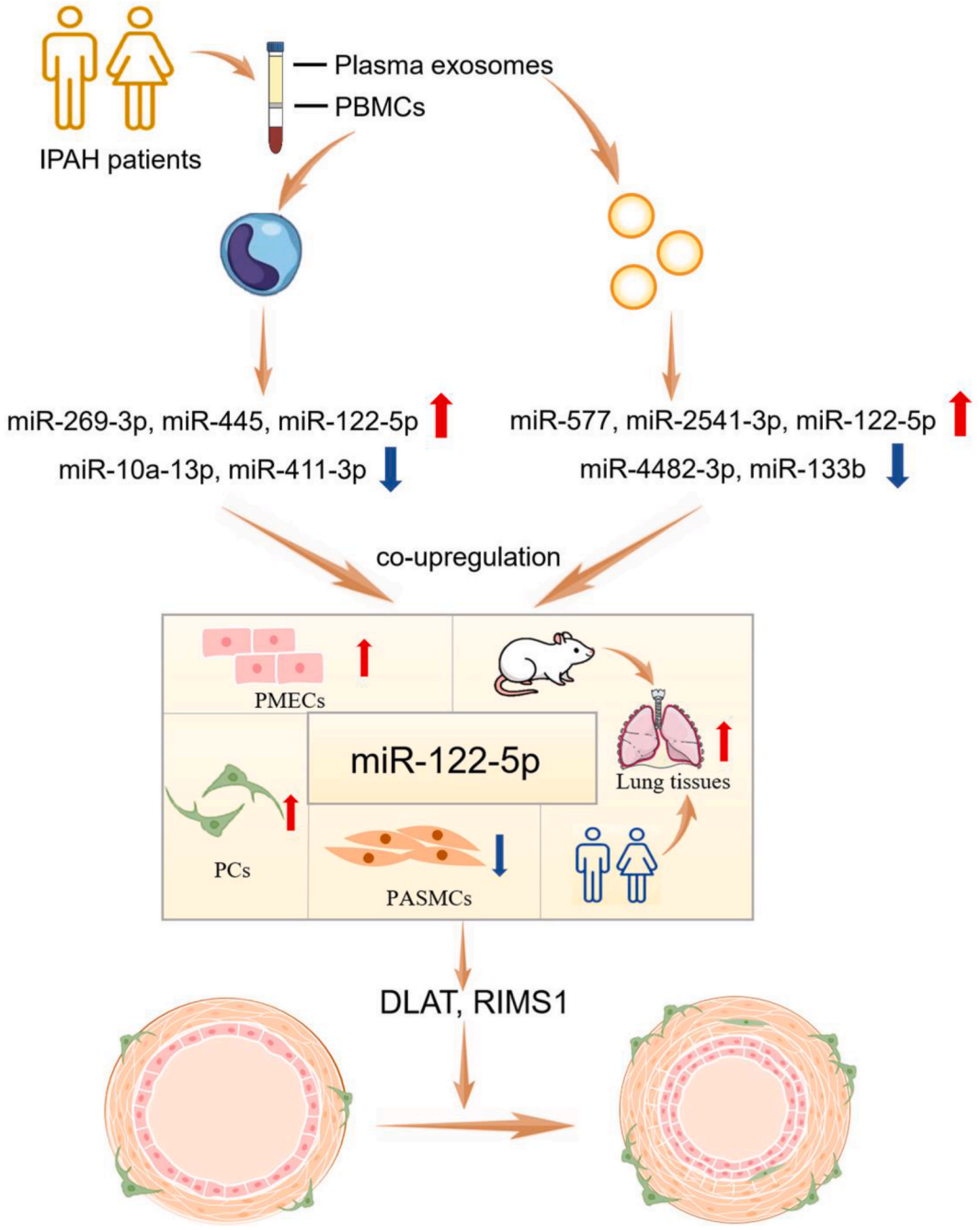

Fig. 7. Coupregulation of miR-122-5p analyzed in plasma exosomes and PBMC in IPAH. MiR-122-5p was validated in pulmonary artery cells and lung tissue and found to target DLAT or RIMS1, thereby playing a role in the pathogenesis of IPAH.

has not been previously reported, our results for PMECs and lung tissue were consistent with those in previous research. For example, the expression of miR-122-5p increased in lipopolysaccharide-induced mouse lung tissue and PMECs [25]. In summary, although miR-122-5p has been the subject of previous studies, its role remains unclear.

In the target gene enrichment pathway predicted by miR-122-5p, the first significantly enriched GO terms for BP are "regulation of hydrogen peroxide -induced cell death". This pathway involves cell death, which plays a key role in the pathogenesis of PH. Vascular wall cells, such as PMECs and PASMCs, appear to proliferate excessively and resist apoptosis, which in turn leads to pulmonary vascular remodeling. The miRNA gene targeting network produced in this study revealed the relationship among differentially expressed miRNAs in plasma exosomes and PBMC of patients with IPAH [37]. Our results suggested that DLAT and RIMS1 may be potential target genes for IPAH. DLAT exists in the inner membrane of mitochondria and plays a role in the breakdown of pyruvate into

Acetyl CoA [38], and RISM1 is a member of ras gene superfamily that regulates synaptic vesicle exocytosis [38]. Although neither of these genes has been studied in relation to IPAH, DLAT imbalance is associated with cell proliferation and migration [38], and RIMS1 is differentially expressed in cancer and associated with cancer prognosis [39,40]. In the GSE117261 database and predicted differentially expressed genes in PMECs, DLAT was downregulated in IPAH and control mRNA samples, whereas RIMS1 was downregulated in hypoxic PMECs. These findings were consistent with our results, confirming that miR-122-5p potentially regulates the pathogenesis of IPAH through DLAT and RIMS1.

We recognize that this study is subject to certain limitations. Firstly, the sample size utilized in this research was relatively small, potentially resulting in insufficient statistical power. Thus, in future studies across multiple centers, the sample size must be increased. Second, our study does not include mechanistic research, which will be conducted as a next step. Third, only patients with IPAH were included in this study, whereas PH subtypes were not included. So, other types of PH samples will be studied in our following research.

5. Conclusion

In conclusion, miR-122-5p was found to be co-upregulated in the PBMC and plasma exosomes of patients with IPAH. Moreover, miR-122-5p was upregulated in hypoxic PMECs and PCs and lung tissue of patients with IPAH and rats, but downregulated in hypoxic PSMACs. Therefore, miR-122-5p may be involved in different biological functions in the adjacent vascular wall cells of IPAH. Finally, miR-122-5p was predicted to target DLAT and RIMS1. Thus, this miRNA may function through these genes as a regulator of vascular wall cells in IPAH.

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Data accessibility statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. All the data generated by this workflow will be submitted to Science Data Bank repository and will be publicly accessible. All working data have already been submitted and are available at this link: https://www.scidb.cn/s/biyyQj. The DOI is: 10.57760/sciencedb.01807.

Data availability statement

Data associated with this study has been deposited at link: https://www.scidb.cn/s/biyyQj. The DOI is: https://doi.org/10.57760/sciencedb.01807.

CRediT authorship contribution statement

Hui Zhao: Data curation, Visualization. **Ruowang Duan:** Formal analysis, Software, Writing – original draft. **Qian Wang:** Formal analysis, Software, Validation. **Xiaoyi Hu:** Investigation, Validation. **Qinhua Zhao:** Data curation, Formal analysis. **Wenhui Wu:** Methodology, Validation. **Rong Jiang:** Software, Supervision. **Sugang Gong:** Formal analysis, Writing – original draft, Writing – review & editing. **Lan Wang:** Project administration, Resources, Writing – review & editing. **Jinming Liu:** Funding acquisition, Resources, Writing – review & editing. **Jie Deng:** Writing – original draft, Writing – review & editing. **Huazheng Liang:** Conceptualization, Resources, Writing – original draft. **Yuqing Miao:** Conceptualization, Project administration, Resources, Writing – review & editing. **Ping Yuan:** Conceptualization, Funding acquisition, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22922.

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