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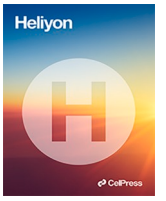

Research article

Endothelial downregulation of nuclear m6A reader YTHDC1 promotes pulmonary vascular remodeling in sugen hypoxia model of pulmonary hypertension

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ABSTRACT

Background: Pulmonary hypertension (PH) is characterized with vascular remodeling, which is intiated by vascular endothelial dysfunction. N6-methyladenosine (m6A) modification mediates gene expression in many ways including mediating RNA degradation, splicing, nuclear export et al. m6A modification have been found to be associated with the development of PH. However, the role of m6A regulators in pulmonary artery endothelial cells (PAECs) dysfunction of PH is still under research.

Methods: The expression levels of m6A regulators in PAECs were analyzed with the single-cell sequencing Data(scRNA). Next, the target differentially expressed genes (DEGs) of m6A regulators in PAECs were functionally annotated. The analysis of cellular interactions included the examination of receptor—ligand pairs regulated by m6A regulators. Pseudo-time trajectory analyses and a ceRNA network involving lncRNAs, miRNAs, and mRNAs were conducted in PAECs. Furthermore, microarray data (GSE180169) for Sugen Hypoxia PH (SuHx PH) mouse models was screened for DEGs and m6A regulators in PAECs. Moreover, the expression of YTHDC1 in the lung samples of SuHx PH models was determined using immunofluorescence. In vitro, the mRNA expression of YTHDC1 in HPAECs under hypoxia conditions was detected. The effect of YTHDC1 recombinant protein on HPAEC proliferation was detected by Cell Counting Kit-8 (CCK8).

Results: Dysregulation of m6A regulators was observed in mouse PAECs. The m6A reader of YTHDC1 was decreased in PAECs in scRNA data and RNAseq data of isolated PAECs of SuHx PH models. Downregulation of YTHDC1 was caused by hypoxia in PAECs in vitro and similar results was observed in PAECs of SuHx PH mouse models. Next, YTHDC1 recombinant protein was found to inhibit HPAECs proliferation. The DEGs targeted by YTHDC1 were enriched in angiogenesis, endothelial cell migration, fluid shear stress, and stem cell maintenance. Analysis indicates that interactions among endothelial cells, smooth muscle cells, fibroblasts, and immune cells, mediated by specific YTHDC1 target genes (e.g., PTPRC-MRC1, ITBG2-ICAM1, COL4A1-CD44), contribute to PH development. Also, the YTHDC1 expression were consistent with Thioredoxin interacting protein (TXNIP). What's more, the predicted transcription factors showed that NFKB1, Foxd3 may be involved in the regulation of YTHDC1. Lastly, our data suggest that YTHDC1 may be involved in regulating PAECs dysfunction through lncRNA/miRNA/mRNA network.

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Conclusion: For the first time, we analyzed changes in the expression and biological functions of m6A regulators in SuHx PH mouse models. We causatively linked YTHDC1 to PAECs dysfunction, providing novel insight into and opportunities to diagnose and treat PH.

1. Introduction

Pulmonary hypertension (PH) is characterized by pulmonary artery vasoconstriction, peripheral vascular inflammation, small arteries obstruction, resulting in right heart failure and ultimately death due to limited treatment [1,2]. Vascular remodeling starts with dysfunction of pulmonary artery endothelial cells (PAECs), including over proliferation, migration and apoptosis resistance [3,4].

N6-methyladenosine (m6A) is the most ubiquitous and richest RNA modification in cells. Current researches have found m6A engagement in a variety of physiological and pathological processes via mRNA transport, degradation, and translation [5].Acting as a reversible modification, m6A methyltransferases (writers), m6A demethylases (erasers), and m6A binding proteins (readers) are the three components involved in the regulation of m6A modification, which participates in various biological processes [6].Dysregulated m6A is involved in tumor, inflammation, metabolic and vascular diseases [7,8].Growing evidence has indicated that the continuous dynamic modulation of m6A as a novel mediator, exerting an effect on specific gene expression and pathophysiological processes of PH [9–11].

At present, several papers have proved that altered m6A modification is involved in PASMCs proliferation and PH development in an m6A-dependent manner [9,11,12]. Increased methylated coding genes in lung samples were associated with inflammation, glycolysis, ECM-receptor interaction and PDGF signal pathway, in addition, decreased methylation genes were related to TGF-β family receptor members [13]. Nonetheless, the roles of m6A regulators in PH have not been fully elucidated. It is particularly critical to further unveiling the mechanism of m6A regulator function and exploring the m6A regulatory network in specific cell type. Especially, the research of m6A regulators in endothelial cells is critical for mechanism exploration and m6A-targeting treatment in PH.

To our knowledge, the study of m6A regulators have not been done in PAECs. In this study, we found that m6A regulators were significantly differently expressed using a scRNA sequencing data and RNAseq data of isolated PAECs. The expression of YTHDC1 in lung samples of SuHx PH mouse models and the effect of YTHDC1 on PAECs proliferation under hypoxia conditions were determined. We also determined functions of differentially expressed genes (target DEGs of YTHDC1) in PAECs. The role of YTHDC1 in cellular interactions was investigated. In addition, we built a network of ceRNA (YTHDC1-lncRNA/miRNA/mRNA) regulatory network and predicted the regulation of YTHDC1 by transcription factors (TFs) in PAECs, which may facilitate identification of biomarkers for PH treatment and diagnosis.

2. Materials and methods

2.1. Data collection

In this study, the scRNA expression data (exprMatrix.tsv) and cell clustering information (meta.tsv) of six controls and six SuHx PH lung samples were downloaded. Bulk RNA-seq profiling of murine endothelial cells of SuHx PH were obtained from the GEO database (gse 180169).

2.2. Difference analysis

We used limma v3.42.2 [14]for differential analysis, screen genes with p-value <0.05, and $|\log2 \text{ (fold change)}| > 0.322$ for downstream analysis, and use ggplot2 v3.3.4 [15] for correlation graphics.

2.3. Association between DEGs and m6A targets

We downloaded the target genes of m6A regulators from m6A2 Target (http://m6A2target.canceromics.org/#/download). Overlapping genes of DEGs and m6A targeted genes were identified.

2.4. Function enrichment analysis

We used clusterProfiler v3.14.3 [16] to perform GO and KEGG functional enrichment analysis on differential genes. The P-values were calculated based on the cumulative hypergeometric distribution.

2.5. Pseudo-timing analysis and cell communication

Cellphonedb v3.0.0 [17] was used for cell communication analysis and monocle v2.14.0 [18]for pseudo-temporal analysis.

2.6. YTHDC1-lncRNA/miRNA/mRNA interaction prediction

The association between YTHDC1 and lncRNA was obtained by RNAct [19]. Prediction of miRNAs interacting with lncRNAs by miRanda [20] (v3.3a). miRNA-mRNA relationship analysis by RNAInter (v3.0) database [21]. Graphing the interaction network by Cytoscape [22].

2.7. Association between target genes and TFs

TFs regulating YTHDC1 are predicted by the R package JASPAR2020 [23] and TFBSTools [24] (non-default parameter: relScore = "85 %").

2.8. Sugen hypoxia PH model and experimental design

The pathophysiology of Sugen hypoxia PH model is consistent with the human PH. The cooperation of sugen SU5416 with chronic hypoxia condition profoundly exacerbated all measures of PAH-like pathology when compared with hypoxia alone. The changes in pulmonary vascular and right heart remodeling in response to hypoxia were further enhanced on SU5416 treatment.

Animals were housed at 24 °C in a 12-h light–dark cycle. Food and water were accessible ad libitum. Twelve 6-week-old male C57/Bl6 mice (20 ± 2.5 g) were divided into the control and pulmonary hypertension group, six mice in each group. The animal model of PH was established by injecting with sugen (sugen SU5416; Sigma-Aldrich) (20 mg/kg weeky) intraperitoneally and the hypoxic chamber was flushed with a mixture of room air and N2, then recirculated (50 % humidity, 5 % CO2, and 24 °C). In addition, control mice received normal saline (0.9 % NaCl). Ethical approval for this investigation was obtained from the Research Ethics Committee, TongJi University School of Medicine.

2.9. Immunofluorescence

Twelve mice were divided into two groups: the control group and the PH group, with six mice in each. The animal model of PH was induced by intraperitoneal injection of sugen (20 mg/kg weekly) into 6-week-old male C57 mice, while the control group received normal saline (0.9 % NaCl). After 4 weeks, lung tissues from the mice were fixed with paraformaldehyde, and YTHC1 expression (1:150, Abcam lot: 31,645) was evaluated through immunofluorescence staining.

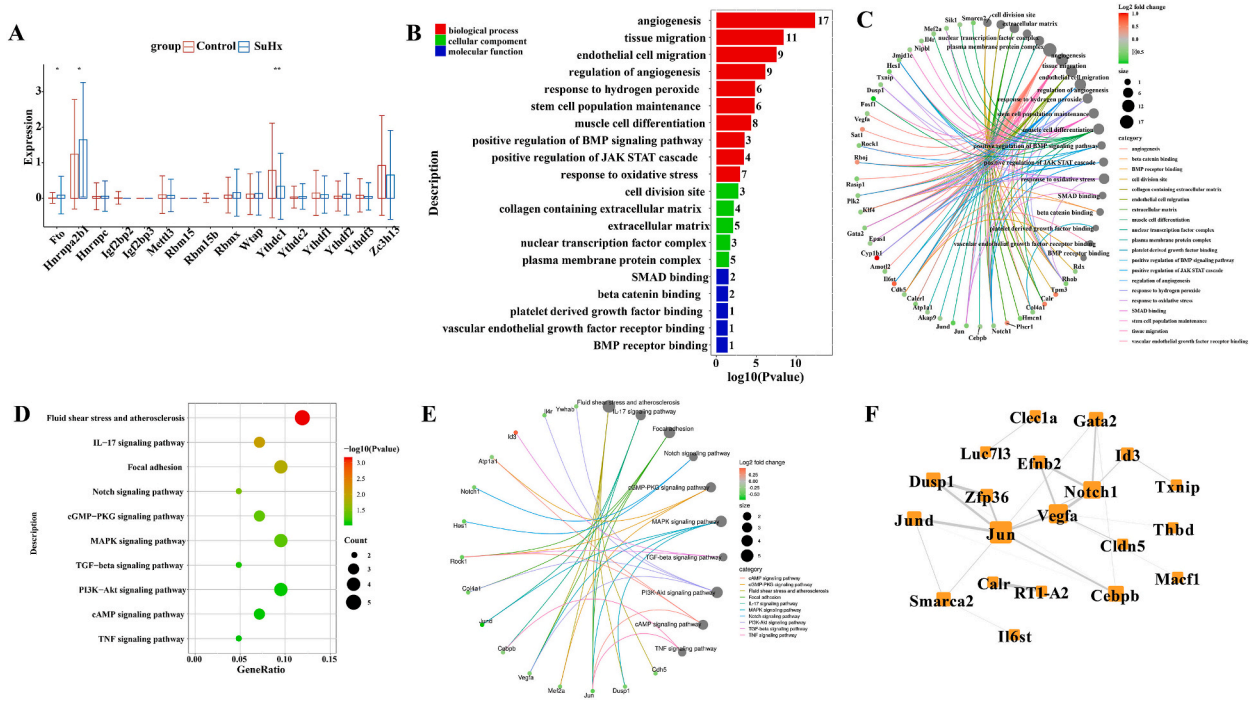

Fig. 1. N6-Methyladenosine regulators expression were dysregulated in PAECs. (A) m6A modulators were differently expressed in PAECs. (B–C) GO analysis of overlapped YTHDC1 target genes with the upregulated DEGs(B), genes in GO enriched pathways(C). (D–E) KEGG analysis of overlapped YTHDC1 target genes with the upregulated DEGs(D), genes in KEGG enriched pathways (E). (F) Hub networks based on genes in enriched pathways.

2.10. Quantitative real-time PCR

Cellular total RNA was extracted utilizing Trizol reagent (Ambion), followed by cDNA synthesis using reverse transcriptase (Thermo Scientific™). Subsequently, RT-PCR was conducted with SYBR Green master mix (Thermo Scientific™) in accordance with the manufacturer's instructions, employing specific primers targeting the corresponding human genes: β-actin forward 5′-CATGGCGGAATTGCTGGTA-3′ and reverse 5′-CGTGCCAACAGCATAGCAGTA-3'; YTHDC1 forward 5′-GGAGGGCCAAATCTCCTACG-3′ and reverse 5′-CTTTTCGGACAGCACGAACG-3'.

2.11. Cell proliferation assessment

HPAECs were seeded in 96 well plates at 1×10^4 cells/well and cultured for 24 h. After serum starved for 24 h, cells were incubated under hypoxia conditions (3 % Oxygen concentration) with human YTHDC1 recombinant protein (Abnova) for 24h. The proliferation of HPAECs was detected by the Cell Counting Kit-8 (Beyotime, China).

2.12. Statistical analysis

Statistical analyses and graphical representations were conducted by GraphPad Prism 8.0 (GraphPad Software). A p-value less than 0.05 was deemed significant. The presented data include the mean ± SEM for each group. Statistical significance for the in vitro data was assessed using the Student t-test for paired data in two distinct experiments.

3. Results

3.1. The expression of N6-Methyladenosine regulators was dysregulated in PAECs

To detect m6A regulators and their function in PAECs of PH, single-cell RNA data was analyzed. Results showed YTHDC1 was dramatically decreased, while FTO and HNRNPA2B1 were significantly upregulated in SuHx PH groups (Fig. 1A). Function of m6A target DEGs was determined by overlapping target genes of YTHDC1 with DEGs. Furthermore, KEGG and GO analysis were performed using overlapped DEGs. The results indicated correlation with angiogenesis, endothelial cell migration, TGFβ signaling, BMP signaling pathway, fluid sheer stress (Fig. 1B and D), genes involving in those pathways were displayed (Fig. 1C and E). Hub networks was also constructed based on genes shown above, including Notch1, VEGFa, etc. (Fig. 1F) .

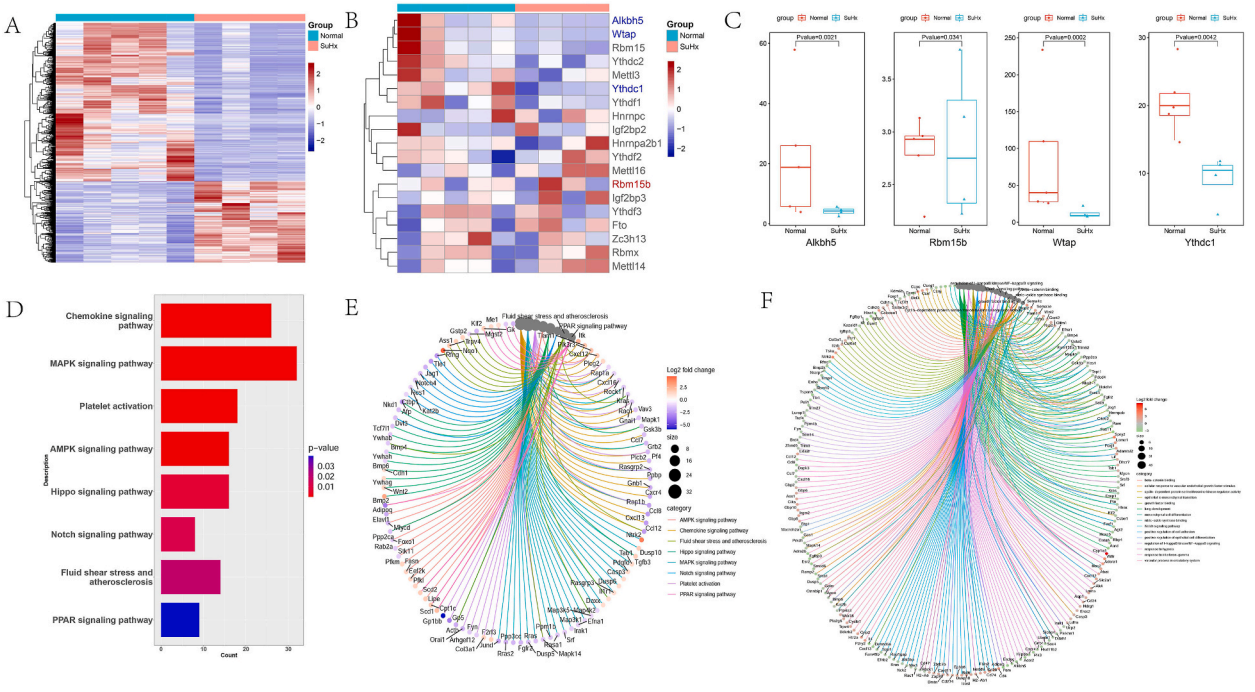

Fig. 2. YTHDC1 was decreased in PAECs isolated from SuHx PH mouse models. (A) The heatmap of differentially expressed genes in the datasets. (B) Different expressions of m6A regulators. (C) Expressions of RBM15b, YTHDC1, ALKBH5 and WTAP in Control and SuHx group. (D) KEGG analysis of m6A target genes overlapped with upregulated genes. (E–F) Genes involved in pathways were presented by KEGG (E) and GO (F) analysis.

3.2. YTHDC1 was decreased in PAECs isolated from SuHx PH mouse models

The study involved exploring the expression of m6A regulators in PAECs isolated from SuHx PH mouse models. Fig. 2A displays a heatmap of the DEGs in PAECs. Validation analysis demonstrated decreased levels of YTHDC1, ALKBH5, and WTAP, and increased levels of RBM15b in PAECs (Fig. 2B and C). Notably, YTHDC1 expression was significantly downregulated in PH model PAECs, consistent with scRNA analysis data (Figs. 1A and 2C). Bioinformatics analysis of overlapped genes between upregulated DEGs and YTHDC1 target genes revealed YTHDC1's involvement in regulating platelet activation, Notch, AMPK signaling, MAPK signaling, Hippo signaling pathways, etc. (Fig. 2D). The genes associated with these functions were detailed in Fig. 2 E-F.

3.3. Downregulation of YTHDC1 was involved in PAECs proliferation

YTHDC1 was downregulated in both lung samples and CD31 positive cells of SuHx PH model compared with controls (Fig. 3 A). In addition, endothelial cell proliferation was increased in HPAECs under hypoxia conditions, while the expression of YTHDC1 was decreased (Fig. 3B and C). Also, the YTHDC1 recombinant protein inhibited the proliferation of HPAECs under hypoxia condition (Fig. 3 D).

3.4. YTHDC1 was involved in vascular remodeling through cellular interaction

Cellular interaction of endothelial cells and smooth muscle cells, fibroblasts, and immune cells involved in vascular remodeling via receptor-ligand pairs, which were regulated by YTHDC1. The resulted indicated that PTPRC-MRC1, ITBG2-ICAM1, were increasing

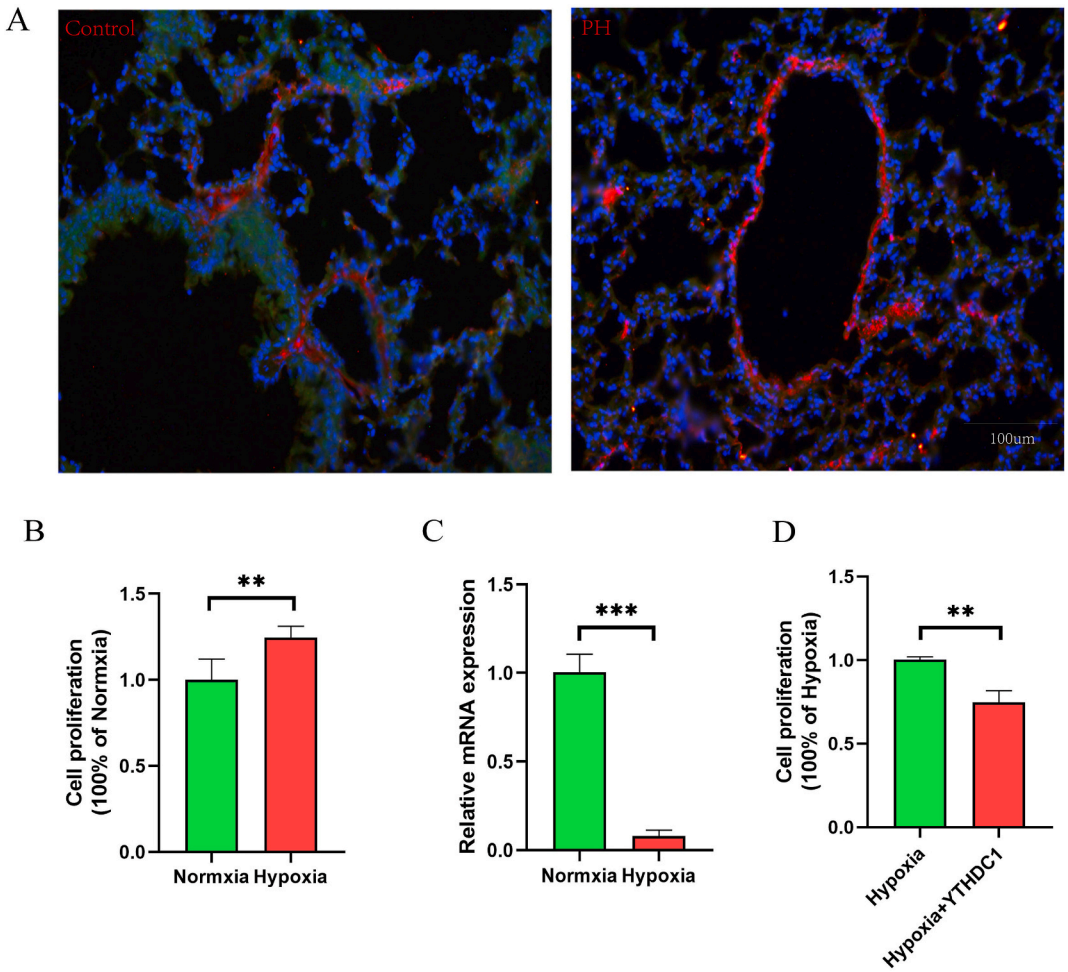

Fig. 3. Endothelial downregulation of YTHDC1 promotes PAECs proliferation. (A) YTHDC1 was downregulated in lung samples and CD31 positive cells of SuHx PH model (right) compared with controls(left), determined by IHC. (B) Proliferation of HPAECs was detected by CCK8 under hypoxia conditions. (C) YTHDC1 was decreased in HPAECs under hypoxia conditions. (D) The effect of YTHDC1 recombinant protein on HPAEC proliferation was detected by CCK8 (*$p < 0.05$,**$p < 0.01$).

between endothelial cells and macrophages (Fig. 4A and B).

3.5. YTHDC1 participated in regulating endothelial cell dysfunction

Pseudo-chronological analysis was conducted with PAECs to uncover distinct characteristics within PAECs subsets. YTHDC1 level was differently expressed in PAECs subsets, suggesting that YTHDC1 involved in the dysfunction of PAECs (Fig. 5A–C). In addition, the expression of YTHDC1 was consistent with the thioredoxin interacting protein (TXNIP) (Fig. 5D), which providing a basis for the mechanism research of YTHDC1 in PAECs.

3.6. YTHDC1-lncRNA/miRNA/mRNA interaction could promote endothelial cell dysfunction

The current study utilized YTHDC1 as a focal point to construct a lncRNA—miRNA—mRNA ceRNA network, providing insights into the regulatory dynamics between YTHDC1 and the ceRNA network and their involvement in pulmonary hypertension (PH) development. Examination of the ceRNA network within pulmonary arterial endothelial cells (PAECs) revealed potential contributions to PAEC dysfunction and pulmonary remodeling, as indicated by combinations like rno-miR-125a-3p/CALR and others (Fig. 6B; Tables 1 and 2). The PPI network of the mRNAs in the ceRNA network showed that NOTCH1/VEGFA signaling might be a downstream mechanism of YTHDC1 (Fig. 6C). The TFs potentially regulating YTHDC1 were predicted, which included NFkB1, Foxd3, and other factors. (Fig. 6A).

4. Discussion

Although the diagnosis and treatment of PH have improved in the past decade, the prognosis of PH patients is still worrisome [25]. A deeper understanding of PH mechanisms contributes to innovative and effectivetreatments [1]. In the past two years, studies have found RNA epigenetic modification, especially m6A methylation modification, plays an important role in the PH occurrence and deterioration [26,27].However, less is known about the effect of m6A regulators in mediating PH-associated vascular remodeling, especially, via regulating endothelial cell dysfunction. We explored the expression profiles in PAECS between SuHx PH mouse models and controls using single-cell sequencing data. The result showed YTHDC1 were largely decreasing in PAECs of PH lung tissues than controls, while RBM15 was significantly upregulated (Figs. 1A–. 2B–C, Fig. 3A and B).

Previous studies reported that m6A regulators participated in endothelial cells dysfunction and excessive proliferation [28,29]. YTHDC1, an important mRNA processing regulator, could regulate mRNA splicing, destabilization and mediating transportation of methylated mRNA from the nucleus to the cytoplasm [30–32]. Researches indicatedYTHDC1 promote tumor progression by targeting MARK signaling via m6A-mediated manner [33,34]. YTHDC1 was also associated with brain injury bypromotes PTEN mRNA degradation and increases AKT phosphorylation [35].In addition, research has shown that YTHDC1 involved in cardiovascular diseases. YTHDC1 promoted aortic dissection development via m6A modification and oxidative stress [36]. Cardiac-specific ablation of YTHDC1 in postnatal heart exhibits progressive dilated cardiomyopathy, heart failure and dramatically increases the incidence of postnatal lethality via regulating TTN splicing, which probably provides a potential target for treating DCM through tuning m6A modification of TTN mRNA [37].

PAECs participated in regulating pulmonary vascular remodeling in a variety of ways, including endothelial-mesenchymalization, cell dysfunction, apoptosis resistance, inflammation, and oxidative stress [38,39]. However, the role of YTHDC1 in the regulation of pulmonary hypertension and PAECs function have not been reported. Functional enrichment analysis of the target genes of YTHDC1

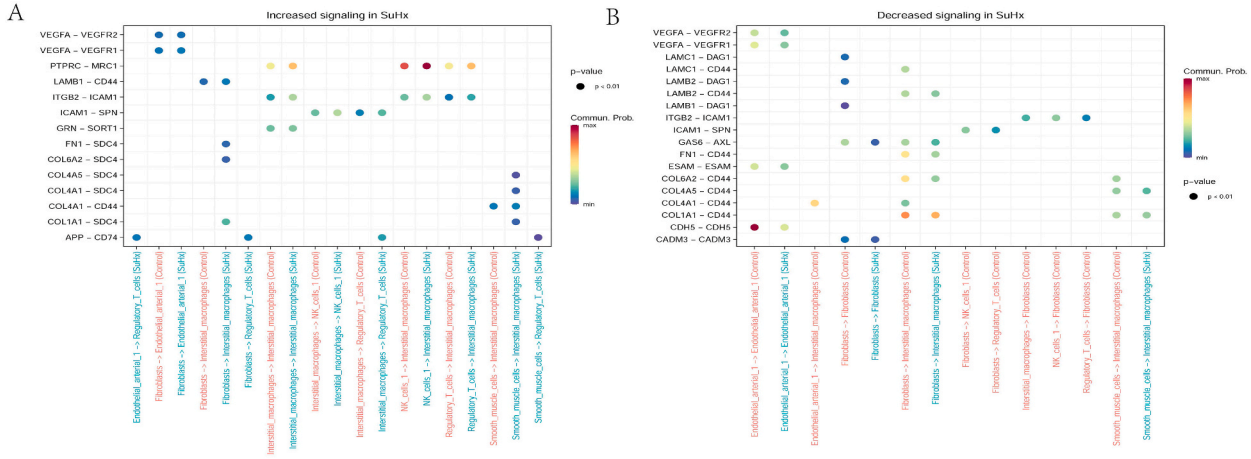

Fig. 4. YTHDC1 participated in regulating PAECs through cellular interaction
(A) Increasing receptor-ligand pairs regulated by YTHDC1 were shown. (B) Decreasing receptor-ligand pairs regulated by YTHDC1 were shown.

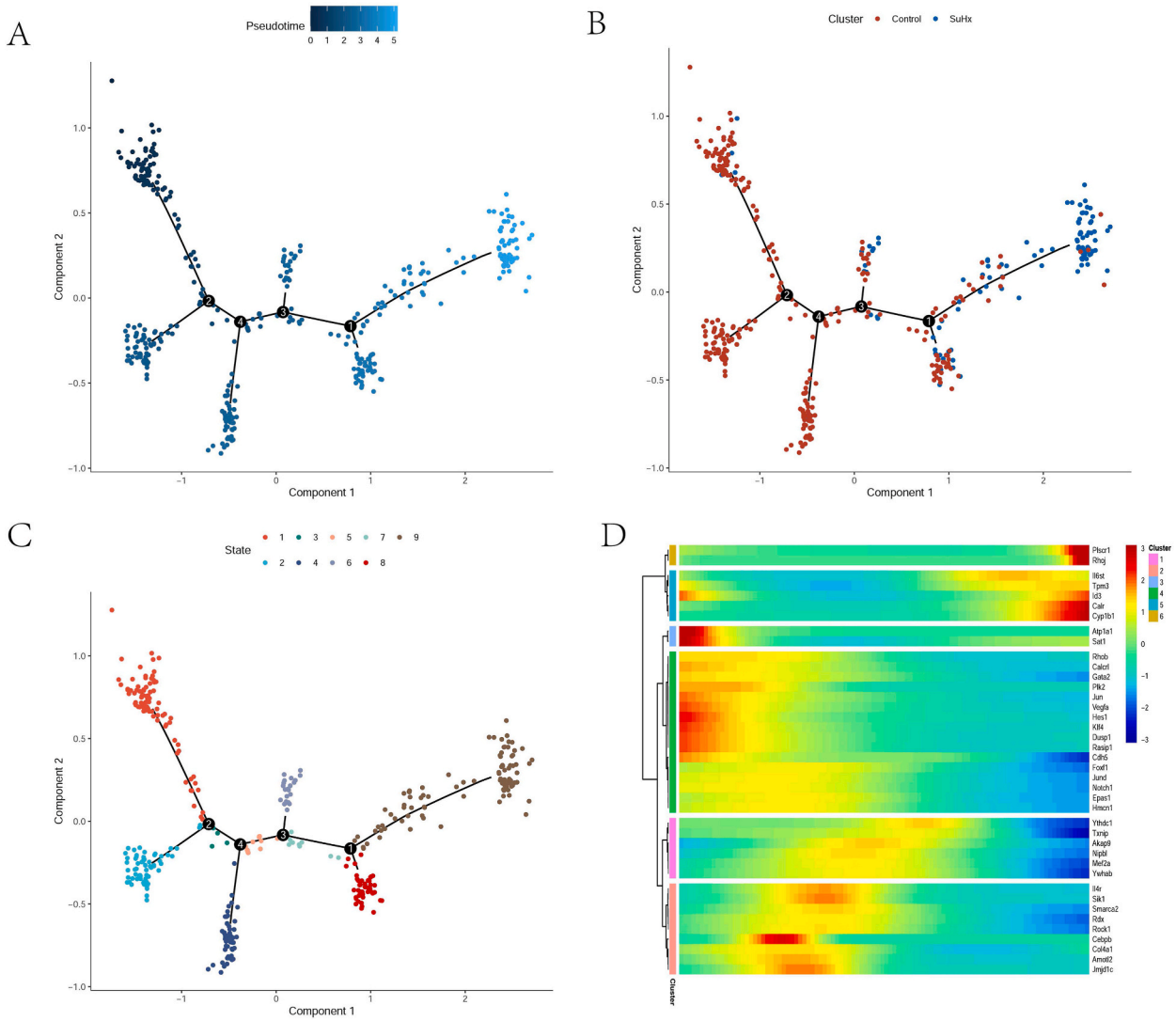

Fig. 5. Results of pseudo-time trajectory analysis conducted with PAECs. (A) Distribution of PAECs followed the pseudo-time trajectory. (B) Cell cluster distributions followed the pseudo-time trajectory with PAECs. (C) Distribution of cell differentiation states along the pseudo-time trajectory. (D)Gene expression revealed in the pseudo-chronological analysis conducted with PAECs.

revealed that YTHDC1 participated in angiogenesis, endothelial cell migration, TGFβ signaling, BMP signaling pathway, fluid sheer stress (Fig. 1B,D), leading to endothelial dysfunction. Moreover, the transcriptome data of PAEC isolated from PH suggested that YTHDC1 involved in platelet activation, Notch, AMPK signaling, MAPK signaling and Hippo signaling pathways (Fig. 2C), mediating endothelial cell injury and pulmonary vascular remodeling. What's more, YTHDC1 were decreasing in HPAECs and the YTHDC1 recombinant protein inhibited the proliferation of HPAECs under hypoxia conditions in vitro (Fig. 3).

To our knowledge, multiple cells were participated in the occurrence and development of PH. However, little is known about receptor-ligand pairs and the regulatory interactions during PH development. It is believed that YTHDC1 could regulate pulmonary artery remodeling through cell interactions via receptor-ligands in a m6A dependent manner. We performed the cellular interactions based on the scRNA dataset and screened the receptor-ligands regulated by YTHDC1. The analysis suggests that PTPRC-MRC1, ITBG2-ICAM1(Fig. 4), were related to interactions between PAECs and macrophages, contributing to exploring new mechanism. m6A modification provides a new direction for the study of endothelial cells and the immune system. Pseudo-chronological analysis revealed different characteristics in PAECs. The results demonstrated a consistency between the expression of YTHDC1 and TXNIP. Studies have indicated that chronic intermittent hypoxia-induced mitochondrial dysfunction contributes to endothelial injury through the TXNIP/NLRP3/IL-1β signaling pathway [40].Also, TXNIP regulating endothelial cells biological functions via oxidative stress and redox systems [40–42]. Redox systems imbalance regulated by m6A modification leads to oxidative stress and mitochondrial dysfunction which may drive the development of endothelial dysfunction [43].

Numerous studies increasingly support the idea that lncRNAs play a role in influencing dysfunction in pulmonary arterial

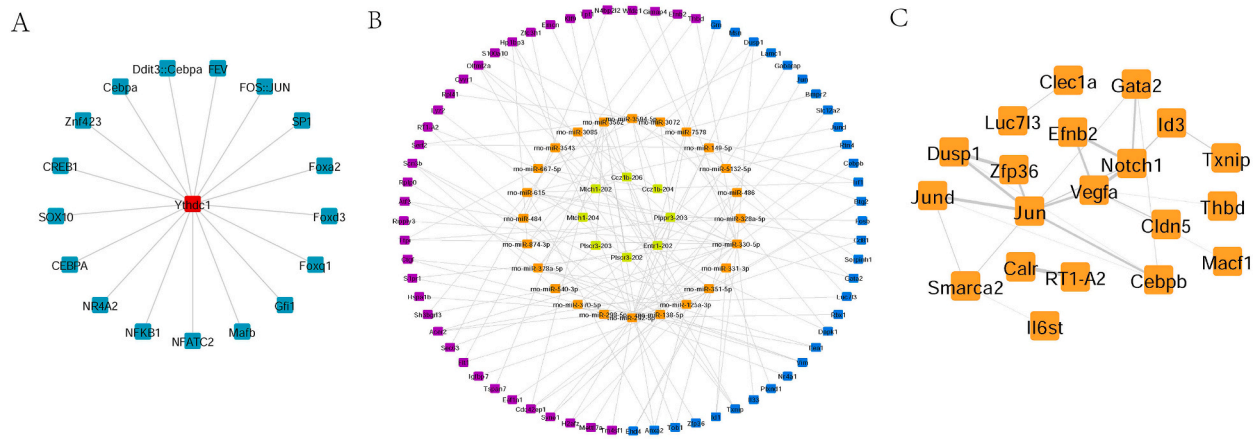

Fig. 6. YTHDC1-lncRNA/miRNA/mRNA interaction promote PAECs dysfunction. (A) Predicted TFs regulating YTHDC1. (B) lncRNA-miRNA-mRNA ceRNA network in PAECs. Yellow, orange nodes represent lncRNAs, miRNAs. Purple and blue nodes represent mRNAs, the later were YTHDC1 target genes. (C) PPI network of the mRNAs in the ceRNA network.

Table 1
The top 5 lncRNAs predicted via YTHDC1 revolving in interaction with miRNAs.

lncRNA	miRNA
Ccz1b-204	rno-miR-3594-5p
Ccz1b-206	rno-miR-484
Entr1-202	rno-miR-125a-3p, rno-miR-149-5p, rno-miR-351-5p, rno-miR-3543, rno-miR-3562, rno-miR-3594-5p
Plppr3-203	rno-miR-138-5p, rno-miR-292-5p, rno-miR-298-5p, rno-miR-3085, rno-miR-328a-5p, rno-miR-330-5p, rno-miR-370-5p, rno-miR-615, rno-miR-7578, rno-miR-874-3p
Plscr3-202	rno-miR-3562

Table 2
mRNAs with their target miRNAs in the ceRNA network.

mRNAs	miRNAs
Acer2	rno-miR-298-5p, rno-miR-330-5p, rno-miR-351-5p
Amotl2	rno-miR-138-5p, rno-miR-149-5p, rno-miR-298-5p, rno-miR-328a-5p, rno-miR-7578
Ankrd12	rno-miR-298-5p
Azin1	rno-miR-125a-3p, rno-miR-138-5p
Calr	rno-miR-125a-3p, rno-miR-328a-5p
Cdc42ep1	rno-miR-125a-3p, rno-miR-330-5p, rno-miR-3562
Cebpb	rno-miR-292-5p
Chd4	rno-miR-370-5p
Cldn5	rno-miR-3594-5p
Clec1a	rno-miR-138-5p, rno-miR-351-5p
Clic5	rno-miR-138-5p, rno-miR-149-5p,rno-miR-3594-5p
Cyp1a1	rno-miR-138-5p
Cyyr1	rno-miR-3085
Dusp1	rno-miR-292-5p, rno-miR-330-5p, rno-miR-874-3p
Efnb2	rno-miR-484

endothelial cells (PAECs) and the remodeling of pulmonary vasculature through various pathways [44–46]. According to the ceRNA hypothesis, the regulatory mechanism involves lncRNAs acting as competitive endogenous RNAs (ceRNAs), influencing gene expression in pulmonary hypertension (PH) through the competition for shared microRNAs (miRNAs) [47,48]. The m6A regulator were involved in the modification of noncoding RNAs [49]. Researches shown that interaction of lncRNA with YTHDC1 driven proliferation and invasion of triple-negative breast cancer [50]. In this study, we predicted the interaction of YTHDC1 with lncRNAs (Fig. 6B–Table 1). Next, we selected the top 5 lncRNAs for analysis. We integrated the interactions between lncRNAs, miRNAs, and mRNAs, constructing a ceRNA network. This network was established to investigate the mechanism of YTHDC1. Results showed that miR-125a-3p involved in regulating cell proliferation and apoptosis [51–53]. Our Result showed that miR-125-3p may contribute to PAECs dysfunction and pulmonary remodeling. In general, by constructing a lncRNA–miRNA–mRNA ceRNA network based on YTHDC1, we identified a regulatory relationship between YTHDC1 and ceRNA network in addition to their roles in PH development.It is suggested that YTHDC1 may modulate the biological processes and pathways of pulmonary arterial endothelial cells (PAECs)

through the lncRNA–miRNA–mRNA ceRNA network. This study enhances our comprehension of the pathogenesis regulated by YTHDC1 and the involvement of the lncRNA–miRNA–mRNA network in the progression of pulmonary hypertension (PH).

In addition, TFs potentially regulating YTHDC1 were predicted in our study (Fig. 6A). Most of TFs are not reported, which require further validation. Nonetheless, we provide fundamental basis for further investigation of YTHDC1 in future studies. Interventions targeting the m6A regulators, ameliorate PH and reduce ECs injury and pathological vascular remodeling will provide new strategies for the diagnosis and treatment of pulmonary arterial hypertension. The mechanisms of YTHDC1 in biological functions offer guidance for future researches and provide potential strategy for clinical diagnose and therapy [54].

In summary, this study firstly reported changes of m6A regulators in PAECs of PH, as well as bioinformatics analysis in PAECs. We reported the crucial roles of YTHDC1 in PAECs dysfunction. Moreover, we verified the decreased expression of YTHDC1 in lung samples of SuHx PH models and HPAECs under hypoxia conditions. Cell interactions via receptor-ligand pairs such as PTPRC-MRC1, ITBG2-ICAM1, COL4A1-CD44, which were target genes of YTHDC1. Notably, the YTHDC1 expression levels were consistent with the TXNIP. In addition, a YTHDC1- lncRNA–miRNA–mRNA ceRNA regulatory network was eastablished in PAECs. Taking together, providing novel insight into and opportunities to diagnose and treat PH.

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Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author. In this study, the scRNA expression data (exprMatrix.tsv) and cell clustering information (meta.tsv) of the samples, which included the gene expression profile of six controls and six SuHx PH lung samples, were downloaded from http://mergeomics.research. idre.ucla.edu/PVDSingleCell/. Bulk RNA-seq profiling of murine endothelial cells of SuHx PH were obtained from the GEO database (gse180169).

CRediT authorship contribution statement

Hao Zheng: Writing – original draft, Project administration, Formal analysis. **Di Wu:** Software, Formal analysis. **Xiangyu Chen:** Writing – review & editing. **Wenjuan He:** Writing – review & editing. **Jing Hua:** Data curation. **Qiang Li:** Funding acquisition, Conceptualization. **YingQun Ji:** Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Qiang Li reports financial support was provided by Top-level Clinical Discipline Project of Shanghai Pudong. Qiang Li reports financial support was provided by National Natural Science Foundation of China. YingQun Ji reports financial support was provided by Key Specialized Construction Project of Shanghai Pudong New Area Health Commission. We would like to thank all the patients who contributed samples for this research. Special thanks for Dear Prof. Jason Hong, from David Geffen School of Medicine at UCLA to share the Single-Cell Analysis Data in PAH animal models(http://mergeomics.research.idre.ucla.edu/PVDSingleCell/). If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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