miRNA-PDGFRB/HIF1A-lncRNA CTEPHA1 Network Plays Important Roles in the Mechanism of Chronic Thromboembolic Pulmonary Hypertension

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Summary

Our previous studies have revealed that long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and genes were abnormally expressed in the pulmonary artery tissues of the chronic thromboembolic pulmonary hypertension (CTEPH) patients. We aim to establish the CTEPH-related miRNA-gene-lncRNA network for finding the core genes and associated miRNA and lncRNA in CTEPH patients.

Firstly, the target genes of differential miRNAs were predicted by searching TargetScan databases, and the predicted target genes were intersected with the mRNAs from the gene chip. Secondly, the intersective genes were analyzed by the Gene Ontology function and Kyoto Encyclopedia of Genes and Genomes pathway software for obtaining differential intersective genes and then establish the miRNA-gene networks. Thirdly, the possible genes regulated by the differential lncRNAs from the gene chip were intersected with the above-screened mRNA to build the lncRNA-mRNA networks. Subsequently, the miRNA-gene-lncRNA networks were constructed according to the two networks above(miRNA-gene networks and lncRNA-mRNA networks). Finally, the core genes of the networks in the experimental group were screened according to Diffk > 0.6 and used to construct the miRNA-core gene-lncRNA networks of CTEPH.

The pathway network, miRNA-mRNA network, lncRNA-mRNA networks, and miRNA-gene-lncRNA networks were successfully constructed. The core genes of the miRNA-gene-lncRNA networks (Diffk > 0.6) were the human Beta-type platelet-derived growth factor receptor (PDGFRB) and hypoxia-inducible factor-1a (HIF-1 A), the miRNAs-PDGFRB-lncRNAs and miRNAs-HIF1A-lncRNAs networks were constructed. Finally, miRNA-149-5p-PDGFRB-TCONS_12_00020587-XLOC_12_010723 and miRNA-338-5p/miRNA-199b-5p-HIF1A-TCONS_12_00020587-XLOC_12_010723 were found in the analysis of the network. miRNA-149-5p-PDGFRB-lncRNA CTEPH-associated 1 (CTEPHA1) (TCONS_12_00020587-XLOC_12_010723) and miRNA-338-5p/miRNA-199b-5p-HIF1A-lncRNA CTEPHA1 are related to the development of CTEPH.

Key words: Gene

Chronic thromboembolic pulmonary hypertension (CTEPH) was classified as group IV of pulmonary hypertension (PH). Persistent pulmonary artery occlusion caused by recurrent embolism is one of the clinical characteristics of CTEPH. Although some indicators can assist in diagnosis, pulmonary angiography is still the gold standard for diagnosis of CTEPH. For the diagnosis of CTEPH, pulmonary endarterectomy is the first choice for treating CTEPH. The lack of treatment will greatly increase the mortality rate in the long run. Some researchers found that inflammation and abnormal pulmonary endothelial cells play a key role in CTEPH, but the concrete mechanism associated with the gene and pathway of CTEPH is not yet clear.

In recent years, much attention has been paid to the long noncoding RNA (lncRNA) due to its function in the gene regulatory network. LncRNA also plays an important role in PH. For example, Sun, et al. reported that lncRNA-maternally expressed gene 3 downregulated human pulmonary artery smooth muscle cell proliferation and migration via the p53 signaling pathway in PH. Another study demonstrated that lncRNA ENSS00000262454 correlated with pericyte markers in idiopathic pulmonary arterial hypertension. In the case of CTEPH, Josipovic, et al. found that lncRNA NONHSAT073641 was upregulated in the vascular samples of pa-
patients with CTEPH and were important for endothelial angiogenic function. Competing endogenous RNAs (ceRNA) was found to lead to the hypothesis that lncRNA could interact with the gene through microRNA (miRNA) by competing for endogenous inhibition.\(^1\) CeRNA has been found in PH.\(^1\) However, the relationships between lncRNA and miRNAs associated with the core genes and the possible mechanism of ceRNA of CTEPH were unclear.

Our previous studies have shown that lncRNAs, miRNAs, and genes were abnormally expressed in the pulmonary artery tissues of the CTEPH patients by microarray.\(^4\)\(^,\)\(^6\) We aim to establish the CTEPH-related miRNA-gene-lncRNA network to identify the most relevant core genes, as well as the related miRNA and IncRNA in CTEPH, which could help to understand the mechanism of CTEPH.

### Methods

The intersective genes 1 obtained by intersecting the predicted target genes of miRNAs with mRNAs genes from gene chip: By searching the database (TargetScan, etc.), all possible target genes regulated by differential 25 miRNAs from the microarray analysis\(^16\) were obtained. Then, we intersected the predicted target genes with the differential 1614 mRNAs from the microarray analysis.\(^16\) The intersective genes were called intersective genes 1.

**GO and KEGG pathway analysis of intersective genes 1:** The Gene Ontology (GO) project provides a controlled vocabulary to describe the attributes of genes and gene products in any organism. The ontology covers three domains: biological processes, cellular components, and molecular functions. Fisher’s exact test in Bioconductor’s top GO package (enrichment analysis for gene ontology) was used to find if there were statistically significant overlaps between the differentially expressed (DE) gene list and the GO-annotated list. The \(P\)-value denotes the significance level of GO-term enrichment in the DE genes. The lower the \(P\)-value, the more significant the GO-term (\(P < 0.05\) is recommended).

Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database that systematically analyzes the relationship between genes, gene functions, and genome information. Based on the KEGG database, significant analysis of the intersective genes 1 in the pathway was made by the Fisher’s exact test and chi-square test, and the significant pathway was obtained by using the \(P\)-value < 0.05.

Taking pathway as the research unit, the pathway interaction network (Pathway-Net) was constructed based on the interaction of differential pathway from KEGG database. When evaluating the pathway in the Pathway-Net diagram, the standard of evaluation is measured by the degree of the pathway in the network. “Degree” represents the number of the relationship between a node and its surrounding nodes in the network. For example, the pathway of its degree of 10 represents a point of 10 pathways interacting with the pathway in the graph. Therefore, the greater the degree, the more interacting pathways can be found.

### Results

**Microarray analysis outcomes:** Our previous studies have found there are 1614 differential mRNA genes (Fold change > 1.2 and \(P\)-value < 0.05), 185 lncRNAs (Fold change > 1.2 and \(P\)-value < 0.05) and 25 miRNAs (Fold change > 2 and \(P\)-value < 0.05) between the experimental and the control group.\(^1\)\(^,\)\(^4\)\(^,\)\(^6\)

**Target genes predicted by miRNA and intersective genes 1:** The number of target genes of 25 miRNAs predicted by miRanda is 10301. We intersected these genes with the 1614 differential mRNA genes from the microar-
Figure 1. GO-Enrichment histogram. Enrichment > 0 indicates the significant increase of GO; Enrichment < 0 indicates the significant decrease of GO. If the $P$-value is the same, the GO with the higher enrichment degree indicates that the greater the influence of the function is, the lower the GO stratification is.
Figure 2. The down-regulated-GO histogram (-LgP). The negative logarithm of P-value (-LgP) and the larger the -LgP value indicated that the smaller the P-value, the higher the GO significant level.

ray analysis. Finally, we obtained 638 intersective genes.

**GO and pathway analysis of intersective genes 1:** As shown in Figures 1-5, the possible functions and pathways of the 638 intersective genes 1 were investigated by GO and pathway analysis. The obviously differential GO-genes were 188 and 44, respectively (P < 0.001), including 252 up and 132 down-regulated GO-genes (P < 0.05), including 159 up and 74 down genes.

The analysis of the interaction network from differential pathways (Pathway-Net): As shown in Figure 6 and Table I, some key pathways in the Pathway-Net are as follows (degree > 10): up pathways: mitogen-activated protein kinase (MAPK) signaling pathway, apoptosis, cytokine-cytokine receptor interaction, p53 signaling pathway; down pathways: Adherens junction; up and down pathways: Pathways in cancer, calcium-signaling pathway, focal adhesion.

**Intersect the GO-genes with the Pathway-genes and construct the miRNA-gene network:** We intersected the 252 up and 132 down-regulated GO-genes with the 159 up and 74 down-regulated pathway-genes to obtain 197 intersective genes 2. We used the 197 intersective genes 2 and the 25 miRNAs from the microarray analysis to construct successfully the 23 miRNAs-197 genes network (Figure 7).

As shown in Tables II, III, the miRNAs that play the most critical role in the network were hsa-miR-149-5p, hsa-miR-138-5p, and hsa-miR-204-5p. In target genes, MLLT4, NFATC2, PRKCA, SGK1, SPN, SSH1, and TRAF1 were often regulated by these miRNAs.

**Construct the lncRNA-gene network:** We used the 185 lncRNAs from the microarray analysis and the 197 intersective genes 2 from the above analysis to construct the 179 lncRNAs-192 genes network according to the adjacent spatial structure of the 185 lncRNA in the experimental and control group, respectively.

As shown in Table IV, the mRNA and lncRNA genes that play the most critical role in the network (DiffK > 0.6): mRNA genes were the human Beta-type platelet-derived growth factor receptor (PDGFRB), hypoxia-
Figure 3. The upregulated-GO histogram (-LgP). The negative logarithm of P-value (-LgP) and the larger the -LgP value indicated that the smaller the P-value, the higher the GO significant level is.
Figure 4. The upregulated-pathway histogram (-LgP). The negative logarithm of P-value (-LgP) and the larger the -LgP value indicated that the smaller the P-value, the higher the pathway significant level is.
Figure 5. The down-regulated-pathway histogram (-LgP). The negative logarithm of P-value (-LgP) and the larger the -LgP value indicated that the smaller the P-value, the higher the pathway significant level is.
Figure 6. The interaction network from differential pathways (Pathway-Net). The circle in the figure represents pathway; the straight line represents the relationship between pathways; red represents the pathway of upregulated gene; blue represents the pathway of down-regulated gene; Yellow represents pathway of both up- and down-regulated genes.

Table 1. Node Properties of Some Key Pathways in the Network (Degree ≥ 10)

| Path_name                        | Style | Degree |
|----------------------------------|-------|--------|
| MAPK signaling pathway           | up    | 30     |
| Apoptosis                        | up    | 20     |
| Pathways in cancer               | all   | 20     |
| Cytokine-cytokine receptor       | up    | 13     |
| Calcium signaling pathway        | all   | 12     |
| Focal adhesion                   | all   | 12     |
| p53 signaling pathway            | up    | 11     |
| Adherens junction                | down  | 10     |

inducible factor-1a (HIF1A), NR4A3, VNN1, and SFRP1. IncRNA genes were NR_022009, NR_030335 and ENST 00000411250.

Construct the miRNA-gene-lncRNA network: As shown in Tables V-VII, in the lncRNA-miRNA-gene co-expression network of the experimental group, genes with important expressive ability (Degree > 12) were MLLT4, SGK1, SFRP1, etc., 16 mRNA genes, lncRNAs (Degree ≥ 15) were TCONS_12_00020587-XLOC_12_010723, NR_030335, TCONS_00014700-XLOC_006798, etc., 15 lncRNAs, miRNAs (Degree ≥ 10) were hsa-miR-149-5p, hsa-miR-138-5p, hsa-miR-204-5p, hsa-miR-199b-5p, etc., 10 miRNAs. In the lncRNA-miRNA-gene co-expression network of the control group, genes with important expressive ability (Degree > 12) were LIFR, PIK3R5, TNXB, etc., 11 mRNA genes, lncRNAs (Degree ≥ 15) were TCONS_00007710-XLOC_003862, NR_026691, NR_027783, etc., 14 lncRNAs, miRNAs (Degree ≥ 10) were hsa-miR-149-5p, hsa-miR-138-5p, hsa-miR-204-5p, hsa-miR-199b-5p, etc., 9 miRNAs.

As shown in Table VIII, the mRNA and lncRNA genes that play the most critical role in the network (DiffK > 0.6): were PDGFRB and HIF1A (mRNA genes) and NR_030335 and TCONS_12_00020587-XLOC_12_010723 (lncRNAs).

Establishment of CTEPH-related miRNAs-PDGFRB-lncRNAs and miRNAs-HIF1A-lncRNAs networks: As
shown in Figure 8, miRNAs related to PDGFRB gene regulation had only miRNA-149-5p. lncRNAs included TCONS_12_00020587-XLOC_12_010723, TCONS_0000111-XLOC_001148, TCONS_12_00009007-XLOC_12_004870, NR_026691, TCONS_12_00021912-XLOC_12_011173, NR_024426, NR_033909, NR_033766, ENST00000411250, TCONS_12_00001868-XLOC_12_001377, NR_022009, and TCONS_12_00004769-XLOC_12_002469. miRNAs related to HIF1A gene regulation had miRNA-388-5p and miRNA-199b-5p; lncRNAs included TCONS_12_00020587-XLOC_12_010723, ENST0000411250, TCONS_12_00014826-XLOC_12_008214, TCONS_12_00015443-XLOC_12_007488, NR_036538, NR_022229, TCONS_12_00004769-XLOC_12_002469, TCONS_12_00023547-XLOC_011380, NR_026691, TCONS_0000111-XLOC_001148, TCONS_12_00009007-XLOC_12_004870, and NR_033766. LncRNAs related to PDGFRB and HIF1A gene regulation included ENST0000411250, TCONS_12_00004769-XLOC_12_002469, NR_026691, TCONS_0000111-XLOC_001148, TCONS

Figure 7. miRNA-Gene network. The corner rectangle in the graph represents miRNA; the circle represents the gene; a straight line represents the regulation relationship between miRNA and the gene; the red mark is upregulated, and the blue mark is down.
Discussion

Several clinical factors have been reported to be significant factors for the occurrence of CTEPH.\textsuperscript{17,18} The pathophysiology of CTEPH remains incompletely understood. As it is difficult to obtain the tissue of the human pulmonary artery, most of the studies on CTEPH are at the animal level.\textsuperscript{19-21} In our previous studies, we performed the gene chip analysis of miRNA, lncRNA, and mRNA using the tissue of pulmonary arteries obtained from the CTEPH patients who underwent pulmonary endarterectomy, and the lung cancer patients who underwent pulmonary lobectomy.\textsuperscript{14-16} However, the relationships between miRNA, lncRNA, and genes were not analyzed. Therefore, a comprehensive network analysis of the three parts based on the chip results was performed in this study.

### Table II. Key miRNAs in the Network (Degree ≥ 10)

| miRNA    | style | Degree |
|----------|-------|--------|
| hsa-miR-149-5p | down | 58     |
| hsa-miR-138-5p | down | 36     |
| hsa-miR-204-5p | down | 34     |
| hsa-miR-199b-5p | down | 16     |
| hsa-miR-10b-5p | down | 14     |
| hsa-miR-125b-2-3p | down | 13     |
| hsa-miR-206 | up    | 13     |
| hsa-miR-3175 | up    | 13     |
| hsa-miR-4525 | up    | 11     |
| hsa-miR-335-5p | down | 10     |

### Table III. The Most Regulated Target Genes in the Network (Degree ≥ 4)

| Gene symbol | Description                                                                 | style | Degree |
|-------------|-----------------------------------------------------------------------------|-------|--------|
| MLLT4       | myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to 4 | down  | 5      |
| NFATC2      | nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 2       | up    | 4      |
| PRKCA       | protein kinase C, alpha                                                        | up    | 4      |
| SGK1        | serum/glucocorticoid regulated kinase 1                                        | up    | 4      |
| SPN         | sialophorin                                                                  | up    | 4      |
| SSH1        | slin/shot homolog 1 (Drosophila)                                              | up    | 4      |
| TRAF1       | TNF receptor-associated factor 1                                               | up    | 4      |

### Table IV. The DiffK > 0.6 Gene/LncRNA in the Network Comparisons of Two Groups

| Gene/lncRNA | Exp_Degree | Exp_K | Con_Degree | Con_K | DiffK (Exp-Con) |
|-------------|------------|-------|------------|-------|-----------------|
| PDGFRB      | 35         | 0.946 | 3          | 0.086 | 0.860           |
| HIF1A       | 35         | 0.946 | 3          | 0.086 | 0.860           |
| NR4A3       | 35         | 0.946 | 5          | 0.143 | 0.803           |
| NR_022009   | 28         | 0.757 | 0          | 0     | 0.757           |
| NR_030335   | 29         | 0.784 | 3          | 0.086 | 0.698           |
| VNN1        | 29         | 0.784 | 4          | 0.114 | 0.669           |
| ENST0000041250 | 29         | 0.784 | 6          | 0.171 | 0.612           |
| SFRP1       | 31         | 0.838 | 8          | 0.229 | 0.609           |

### Table V. The Genes with Important Expressive Ability in the Networks of Two Groups (Degree ≥ 12)

| Gene symbol | E group | Style | Degree | C group |
|-------------|---------|-------|--------|---------|
| MLLT4       | LIFR    | down  | 16     | 15      |
| SGK1        | PIK3R5  | up    | 16     | 15      |
| SFRP1       | TNXB    | down  | 15     | 14      |
| DDX58       | ABLIM1  | up    | 14     | 13      |
| HIF1A       | ANGPT2  | up    | 14     | 13      |
| PTPRC       | BCL2L11 | up    | 14     | 13      |
| FCGR2A      | EDA2R   | up    | 13     | 12      |
| FCGR3A      | CCR4    | up    | 13     | 12      |
| P2RY14      | FCGR3A  | down  | 13     | 12      |
| PDGFRB      | NFATC2  | up    | 13     | 12      |
| SGPL1       | THR     | up    | 13     | 12      |
| ABLIM1      | down    | 12    |        |        |
| DAPP1       | up      | 12    |        |        |
| NR4A3       | up      | 12    |        |        |
| SERP1N9     | up      | 12    |        |        |
| VNN1        | up      | 12    |        |        |
duct polymerase chain reaction (PCR) verification and re-
show that it is associated with CTEPH. We plan to con-
149. However, there has not been any study report to
through p38MAPK-mediated downregulation of miRNA-
pression of genes associated with endothelial dysfunction
PH.22-25) CTEPH is a special type of PH, and if MAPK
plays an important role in the development of
work (Degree = 30). Numerous studies have reported that
MAPK plays the same role in CTEPH, further analysis and verifi-
firstly, in the CTEPH-related pathway network, we
found that the MAPK pathway is at the core of the net-
work (Degree = 30). Numerous studies have reported that
MAPK plays an important role in the development of
CTEPH is a special type of PH, and if MAPK
plays the same role in CTEPH, further analysis and verifi-
network need to be done in the future. Secondly, in our
work, et al.27) found that tumor necrosis factor-
induced the ex-
expression of genes associated with endothelial dysfunction
through p38MAPK-mediated downregulation of miRNA-
However, there has not been any study report to
show that it is associated with CTEPH. We plan to con-
duct polymerase chain reaction (PCR) verification and re-
lated functional analysis of it in the pulmonary arteries
tissue of CTEPH patients in the future. Thirdly, based on
the analysis of the lncRNA-gene network of the experi-
mental and the control group, we found that the
PDGFRB, HIF1A, NR4A3, VNN1, and SFRP1 genes may
play important roles in the development of CTEPH (Diffk
> 0.6). Finally, in the miRNA-gene-lncRNA network, we
screened miRNAs and lncRNAs related to them in the
analysis of the value of Diffk (Diffk > 0.6); then, we
constructed a new network based on this finding (Figure 8).

| Table VI. LncRNA of Degree ≥ 15 in the Networks of Two Groups |
| E group | LncRNA Accession | C group | Style | Degree |
|---------|-----------------|---------|-------|--------|
| TCONS_12_00020587-XLOC_12_010723 | TCONS_00007710-XLOC_003862 | up | C group | 27 | 26 |
| NR_030335 | TCONS_00014700-XLOC_006798 | down | 24 | 24 |
| NR_030335 | NR_027783 | up | 21 | 23 |
| NR_033766 | NR_033494 | down | 20 | 22 |
| NR_024426 | NR_003945 | up | 19 | 21 |
| TCONS_12_00001868-XLOC_12_001377 | ENST00000411250 | down | 19 | 20 |
| NR_036693 | NR_001284 | down | 18 | 18 |
| NR_002956 | TCONS_00028797-XLOC_013868 | up | 17 | 18 |
| NR_026691 | ENST00000499503 | up | 17 | 17 |
| TCONS_12_00004769-XLOC_12_002469 | TCONS_00020410-XLOC_009736 | down | 17 | 17 |
| TCONS_0000111-XLOC_001148 | ENST00000499503 | up | 16 | 16 |
| TCONS_12_00023547-XLOC_011380 | TCONS_0003243-XLOC_002035 | down | 16 | 16 |
| NR_022009 | TCONS_0014700-XLOC_006798 | up | 15 | 15 |
| NR_028408 | TCONS_00027051-XLOC_013093 | down | 15 | 15 |
| NR_036538 | TCONS_12_00004769-XLOC_12_002469 | down | 15 | 15 |

| Table VII. miRNA of Degree ≥ 10 in the Networks of the Two Groups |
| microRNA | E group | C group | Style | Degree |
|----------|---------|---------|-------|--------|
| hsa-miR-149-5p | hsa-miR-149-5p | down | 56 | 56 |
| hsa-miR-138-5p | hsa-miR-138-5p | down | 34 | 36 |
| hsa-miR-204-5p | hsa-miR-204-5p | down | 33 | 34 |
| hsa-miR-199b-5p | hsa-miR-199b-5p | down | 16 | 16 |
| hsa-miR-10b-5p | hsa-miR-10b-5p | down | 13 | 14 |
| hsa-miR-125b-2-3p | hsa-miR-125b-2-3p | down | 13 | 13 |
| hsa-miR-3175 | hsa-miR-3175 | up | 13 | 12 |
| hsa-miR-206 | hsa-miR-206 | up | 11 | 11 |
| hsa-miR-335-5p | hsa-miR-335-5p | down | 10 | 10 |
| hsa-miR-4525 | hsa-miR-4525 | up | 10 | 10 |

| Table VIII. Important Role Genes/LncRNA/miRNA in the Network of Experimental Group (DiffK > 0.6) |
| Gene/LncRNA/miRNA | Exp_Degree | Exp_K | Con_Degree | Con_K | DiffK (Exp-Con) |
|-------------------|-----------|-------|------------|-------|----------------|
| PDGFRB | 13 | 0.813 | 0 | 0.000 | 0.813 |
| HIF1A | 14 | 0.875 | 4 | 0.267 | 0.608 |
| NR_030335 | 24 | 0.889 | 5 | 0.000 | 0.889 |
| TCONS_12_00020587-XLOC_12_010723 | 27 | 1.000 | 8 | 0.308 | 0.692 |
based on our available clinical data.

PDGFRB plays an important role in PH. For example, in the related downstream pathway of PDGFRB, the PDGF-BB/PDGFR genes were associated with the regulation of pulmonary vascular tension through the mechanism of prostaglandin formation, calcium increase, MAPK-or PI3K/Akt/mTOR signal transduction, and actin remodeling activation. NBL1 inhibited the proliferation of human pulmonary vascular smooth muscle cells (PASMC) induced by PDGF-BB through blocking PDGF beta-p38MAPK pathway. In the CTEPH-related Pathway-Net of our study, the MAPK and calcium-signaling pathways were at the core of the network (Degree = 31, 12), which is consistent with the abovementioned report. Therefore, we suspect that PDGFRB also activates the MAPK and calcium-signaling pathway in the CTEPH. Further verification is needed in the future.

In the research about the relationship between PDGFRB gene and lncRNA, it has been reported by Chen et al. that the expression levels of PDGFRB gene were regulated by lncRNA-LNRPT in the animal experiment. However, there were no reports about which lncRNAs regulated the expression of the PDGFRB gene in humans. It was found in our final result that there were 12 lncRNAs related to the regulation of PDGFRB gene, but only TCONS_12_00020587-XLOC_12_010723 was the most closely related to the CTEPH and we named it lncRNA CTEPH-associated 1 (lncRNA CTEPHA1). Therefore, the function of PDGFRB-LncRNA CTEPHA1 related to CTEPH will be verified by the gene knockout and overexpression in future experiments. In addition, for the related drugs, imatinib can relax the capillary resistance of the pulmonary vein bed by antagonizing PDGFRB in PH. This suggests that the development of inhibitors for PDGFRB is very important. The research of the relevant pathways of PDGFRB will be greatly helpful for the development of such inhibitors.

HIF1A plays a key role in PASMCs proliferation induced by PDGF. Animal level studies have found that the deletion of HIF1A and the antagonism of PDGFBB reduced the expression of protease-activated receptors and thus, inhibited the induced pulmonary vascular remodeling in PH. These results show that there may be an association between PDGFRB and HIF-1A in PH. In our final PDGFRB and HIF1A core network, there were 7 lncRNAs involved in PDGFRB and HIF1A. The 7 lncRNAs may be a bridge linking PDGFRB and HIF1A expression. We will further verify our hypothesis in future experiments. HIF1A protein was also expressed in the pulmonary artery and right ventricle of PE rats, suggesting that HIF1A may not only be associated with pulmonary artery remodeling, but also the right ventricular hypertrophy. For the study of HIF1A at the human cell level, hypoxia-induced HIF-1A signal transduction, which results in the dysfunction of human pulmonary artery endothelial cell, and subsequently leads to PH. Whether HIF
1A is related to CTEPH need to be further investigated in the future.

For miRNA and lncRNA related to PDGFRB and HIF1A in the final network, miRNA-149 is negatively related to PDGFRB in the final network, and we assume that its role in CTEPH may be played by negative feedback regulation of the PDGFRB. It has been found that miRNA-199a and miRNA-214 are highly expressed in the rat model of PH, which is associated with heart failure caused by pulmonary arterial hypertension. In our final network, miRNA-199b-5p is negatively correlated with HIF1A in CTEPH. The rest of miRNA and lncRNA related to PDGFRB and HIF1A in the final network are not yet reported to be associated with PH or CTEPH.

Some limitations exist in our study. First, these results were observed only based on CTEPH patients. It was not observed whether other types of PH had the same results. However, based on relevant literature, these networks may also play an important role in other types of PH. In future, we will also study whether other types of PH have the same results. Secondly, we have not verified the pathways of miRNA-149-5p-PDGFRB-lncRNA CTEPHA1 and miRNA-338-5p/miRNA-199b-5p-HIF1A-lncRNA CTEPHA1 in CTEPH by PCR.

Conclusion

In summary, the two pathways of miRNA-149-5p-PDGFRB-lncRNA CTEPHA1 and miRNA-338-5p/miRNA-199b-5p-HIF1A-lncRNA CTEPHA1 may be closely related to the development of CTEPH. The verification experiments will be conducted in the future.

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Disclosure

Conflicts of interest: None.

References

1. Galie N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). Eur Heart J 2016; 37: 67-119.
2. Wada H, Sakakura K, Ito M, et al. Right ventricular stroke work index. Int Heart J 2018; 59: 1047-51.
3. Sugimoto K, Yamada A, Takada K, et al. Usefulness of right ventricular basal free wall strain by two-dimensional speckle tracking echocardiography in patients with chronic thromboembolic pulmonary hypertension. Int Heart J 2015; 56: 100-4.
4. Ieda S, Yoshida T, Yamagata Y, et al. Deeper S wave in lead V leads is clinically useful electrocardiographic parameters for predicting pulmonary hypertension. Int Heart J 2018; 59: 136-42.
5. Mahmud E, Madani MM, Kim NH, et al. Chronic thromboembolic pulmonary hypertension: evolving therapeutic approaches for operable and inoperable disease. J Am Coll Cardiol 2018; 71: 2468-86.
6. Quadry SR, Swift AJ, Billings C, et al. The impact of patient choice on survival in chronic thromboembolic pulmonary hypertension. Eur Respir J 2018; 52: 1800589.
7. Skoro-Sajer N, Gerges C, Gerges M, et al. Usefulness of thrombosis and inflammation biomarkers in chronic thromboembolic pulmonary hypertension-sampling plasma and surgical specimens. J Heart Lung Transplant 2018; 37: 1067-74.
8. Mercier O, Arthur Ataam J, Langer NB, et al. Abnormal pulmonary endothelial cells may underlie the enigmatic pathogenesis of chronic thromboembolic pulmonary hypertension. J Heart Lung Transplant 2017; 36: 305-14.
9. Sun Z, Nie X, Sun S, et al. Long non-coding RNA MEG3 downregulation triggers human pulmonary artery smooth muscle cell proliferation and migration via the p53 signaling pathway. Cell Physiol Biochem 2017; 42; 2569-81.
10. Bischoff FC, Werner A, John D, et al. Identification and functional characterization of hypoxia-induced endoplasmic reticulum stress regulating lncRNA (HypERlnc) in pericytes. Circ Res 2017; 121: 368-75.
11. Josipovic I, Fork C, Preussner J, et al. PAFAH1B1 and the lncRNA NONHSAT073641 maintain an angiogenic phenotype in human endothelial cells. Acta Physiol 2016; 218: 13-27.
12. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfo PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 2011; 146; 355-8.
13. Zhuo Y, Zeng Q, Zhang P, Li G, Xie Q, Cheng Y. Functional polymorphism of IncRNA MALAT1 contributes to pulmonary arterial hypertension susceptibility in Chinese people. Clin Chem Lab Med 2017; 55: 38-46.
14. Gu S, Li G, Zhang X, et al. Aberrant expression of long non-coding RNAs in chronic thromboembolic pulmonary hypertension. Mol Med Rep 2015; 11: 2631-43.
15. Gu S, Su P, Yan J, et al. Comparison of gene expression profiles and related pathways in chronic thromboembolic pulmonary hypertension. Int J Mol Med 2014; 33: 277-300.
16. Guo L, Yang Y, Liu J, et al. Differentially expressed plasma microRNAs and the potential regulatory function of Let-7b in chronic thromboembolic pulmonary hypertension. PloS one 2014; 9; e101055.
17. Ende-Verhaar YM, Cannegieker SC, Vonk Noordegraaf A, et al. Incidence of chronic thromboembolic pulmonary hypertension after acute pulmonary embolism: a contemporary view of the published literature. Eur Respir J 2017; 49; 1601792.
18. Sada R, Tanabe N, Ishida K, et al. Prognostic and pathophysiological marker for patients with chronic thromboembolic pulmonary hypertension: usefulness of diffuse density index for carbon monoxide at diagnosis. Respiratoryology 2017; 22; 179-86.
19. Deng C, Zhong Z, Wu D, et al. Role of FoxO1 and apoptosis in pulmonary vascular remodeling in a rat model of chronic thromboembolic pulmonary hypertension. Sci Rep 2017; 7; 2270.
20. Neto-Neves EM, Brown MB, Zaretskaia MV, et al. Chronic embolic pulmonary hypertension caused by pulmonary embolism and vascular endothelial growth factor inhibition. Am J Pathol 2017; 187; 700-12.
21. Deng C, Wu D, Yang M, et al. The role of tissue factor and autophagy in pulmonary vascular remodeling in a rat model for chronic thromboembolic pulmonary hypertension. Respir Res 2016; 17; 65.
22. Maruyama H, Dewachter C, Belhaj A, et al. Endothelin-Bone morphogenetic protein type 2 receptor interaction induces pulmonary artery smooth muscle cell hyperplasia in pulmonary arterial hypertension. J Heart Lung Transplant 2015; 34; 468-78.
23. Shi R, Wei Z, Zhu D, et al. Baicalin attenuates monocrotaline-induced pulmonary arterial hypertension by inhibiting vascular remodeling in rats. Pulm Pharmacol Ther 2018; 48: 124-35.

24. Kojonazarov B, Novoyatleva T, Boehm M, et al. p38 MAPK inhibition improves heart function in pressure-loaded right ventricular hypertrophy. Am J Respir Cell Mol Biol 2017; 57: 603-14.

25. Gao L, Liu J, Hao Y, et al. Chronic intermittent hypobaric hypoxia attenuates monocrotaline-induced pulmonary arterial hypertension via modulating inflammation and suppressing NF-kappaB/p38 pathway. Iran J Basic Med Sci 2018; 21: 244-52.

26. Chamorro-Jorganes A, Araldi E, Rotllan N, Cirera-Salinas D, Suarez Y. Autoregulation of glypican-1 by intronic microRNA-149 fine tunes the angiogenic response to FGF2 in human endothelial cells. J Cell Sci 2014; 127: 1169-78.

27. Palmieri D, Capponi S, Geroldi A, Mura M, Mandich P, Palombo D. TNFalpha induces the expression of genes associated with endothelial dysfunction through p38MAPK-mediated down-regulation of miR-149. Biochem Biophys Res Commun 2014; 443: 246-51.

28. Rieg AD, Suleiman S, Anker C, et al. PDGF-BB regulates the pulmonary vascular tone: impact of prostaglandins, calcium, MAPK- and PI3K/AKT/mTOR signalling and actin polymerisation in pulmonary veins of guinea pigs. Respir Res 2018; 19: 120.

29. Cui C, Zhang H, Guo LN, et al. Inhibitory effect of NBL1 on PDGF-BB-induced human PASMC proliferation through blockade of PDGFbeta-p38MAPK pathway. Biosci Rep 2016; 36: e00374.

30. Chen J, Guo J, Cui X, et al. The long noncoding RNA LnRPT is regulated by PDGF-BB and modulates the proliferation of pulmonary artery smooth muscle cells. Am J Respir Cell Mol Biol 2018; 58: 181-93.

31. Leong ZP, Okida A, Higuchi M, Yamano Y, Hikasa Y. Reversal effects of low-dose imatinib compared with sunitinib on monocrotaline-induced pulmonary and right ventricular remodeling in rats. Vasc Pharmacol 2018; 100: 41-50.

32. Sheikh AQ, Sadosuk FZ, Nokou A, Mazurek R, Greif DM. Cell autonomous and non-cell autonomous regulation of SMC progenitors in pulmonary hypertension. Cell Rep 2018; 23: 1152-65.

33. Kumar S, Wang G, Liu W, et al. Hypoxia-induced mitogenic factor promotes cardiac hypertrophy via calcium-dependent and hypoxia-inducible factor-1alpha mechanisms. Hypertension 2018; 72: 331-42.

34. Liu W, Zhang Y, Lu L, Wang L, Chen M, Hu T. Expression and correlation of hypoxia-inducible factor-1alpha (HIF-1alpha) with pulmonary artery remodeling and right ventricular hypertrophy in experimental pulmonary embolism. Med Sci Monit 2017; 23: 2083-8.

35. He M, Ma S, Cai Q, et al. Hypoxia induces the dysfunction of human endothelial colony-forming cells via HIF-1alpha signaling. Respir Physiol Neurobiol 2018; 247: 87-95.

36. Stevens HC, Deng L, Grant JS, et al. Regulation and function of miR-214 in pulmonary arterial hypertension. Pulm Circ 2016; 6: 109-17.