Prediction of target genes for miR-140-5p in pulmonary arterial hypertension using bioinformatics methods

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The expression of microRNA (miR)-140-5p is known to be reduced in both pulmonary arterial hypertension (PAH) patients and monocrotaline-induced PAH models in rat. Identification of target genes for miR-140-5p with bioinformatics analysis may reveal new pathways and connections in PAH. This study aimed to explore downstream target genes and relevant signaling pathways regulated by miR-140-5p to provide theoretical evidences for further researches on role of miR-140-5p in PAH. Multiple downstream target genes and upstream transcription factors (TFs) of miR-140-5p were predicted in the analysis. Gene ontology (GO) enrichment analysis indicated that downstream target genes of miR-140-5p were enriched in many biological processes, such as biological regulation, signal transduction, response to chemical stimulus, stem cell proliferation, cell surface receptor signaling pathways. Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis found that downstream target genes were mainly located in Notch, TGF-beta, PI3K/Akt, and Hippo signaling pathway. According to TF–miRNA–mRNA network, the important downstream target genes of miR-140-5p were enriched in many biological processes, such as biological regulation, signal transduction, response to chemical stimulus, stem cell proliferation, cell surface receptor signaling pathways. Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis found that downstream target genes were mainly located in Notch, TGF-beta, PI3K/Akt, and Hippo signaling pathway. According to TF–miRNA–mRNA network, the important downstream target genes of miR-140-5p were PPI, TGF-betaR1, smad4, JAG1, ADAM10, FGF9, PDGFRA, VEGFA, LAMC1, TLR4, and CREB. After thoroughly reviewing published literature, we found that 23 target genes and seven signaling pathways were truly inhibited by miR-140-5p in various tissues or cells; most of these verified targets were in accordance with our present prediction. Other predicted targets still need further verification in vivo and in vitro.

Pulmonary arterial hypertension (PAH) is a chronic progressive disease of pulmonary vasculature characterized by sustained elevation of pulmonary vascular resistance and pulmonary arterial pressure, consequently leading to right heart failure and eventual death [1]. The pathogenesis of PAH is associated with genetic predisposition, inflammation, increase in vascular tone, elevation in pulmonary artery cell proliferation and resistance to apoptosis, and the presence of in situ thrombosis [2–5]. Effect of current treatment on PAH remains poor and available therapies to improve long-term prognosis are limited [6], so exploring novel molecular mechanisms and generating therapeutic approaches are urgently needed. MicroRNAs (miRNAs) are small noncoding RNA molecules around 22 nucleotides long that bind the 3’-untranslated region (UTR) of mRNA to degrade mRNA and therefore to negatively regulate relevant genes expression [7].

Abbreviations
GO, gene ontology; KEGG, kyoto encyclopedia of genes and genome; PAH, pulmonary arterial hypertension; PASMC, pulmonary arterial smooth muscle cell; TF, transcription factor.
miRNAs have the ability to target numerous genes mRNA, therefore potentially controlling a host of genes expression and the activity of multiple signaling pathways [8–10]. Recent studies have shown that reduction in microRNA (miR)-140-5p is found in both patients with PAH and monocrotaline-induced PAH models in rat, which is involved in the development of PAH [11,12]. Therefore, it is important to identify comprehensive downstream targets of miR-140-5p with bioinformatics analysis in PAH, and this might provide some critical information for the development and treatment of PAH. In this study, downstream target genes regulated by miR-140-5p and upstream transcription factors (TFs) regulating miR-140-5p expression were predicted, and the downstream target genes were analyzed for gene ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genome (KEGG) pathway. Next, the upstream TFs and downstream targets of miR-140-5p were determined according to the TF–miRNA–mRNA network. Finally, the direct downstream targets and relevant signaling pathways regulated by miR-140-5p were obtained in published literature and were compared with the predicted results of this study.

Materials and methods

Mature sequences of miR-140-5p in various species

Mature sequences of miR-140-5p in various species were obtained in the miRBase database (http://mirbase.org/index.shtml).

Target gene prediction of miR-140-5p

Identification of target genes is critical for characterizing the functions of miRNAs. In this study, miRanda (http://www.microrna.org/), TargetScan (http://www.targetscan.org/), RNAhybrid (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/submission.html), and miRDB (http://www.mirdb.org/) databases were used to predict the target genes of miR-140-5p. To make our predicted target genes more convincible, only the target genes predicted by at least three databases were selected for further analyses.

Database-based GO and KEGG pathway enrichment analysis

Target mRNA of miR-140-5p supported by at least three databases were used for GO analysis to predict gene functions. Integration Discovery (DAVID) software, version 6.7 (http://david.abcc.ncifcrf.gov), was used to perform GO analysis to identify biological processes, cellular components, and molecular functions of these target genes. At the same time, the probable signaling pathways in which these target genes were enriched were analyzed by KEGG database (http://www.genome.jp/kegg/). The P-value <0.05 was considered significant.

Upstream TFs prediction of miR-140-5p

Human miR-140-5p precursor was obtained in the miRBase database and its 5000 bp upstream was defined as the miR-140-5p promoter. The TFs of miR-140-5p were predicted using MOODS-python software (version 1.9.3) in JASPAR database (http://jaspar.binf.ku.dk/), which includes various vertebrate TFs. The P-value <0.0001 was considered significant.

Construction of the network for TF–miR-140-5p–mRNA

By merging the regulatory relationships between TFs and miR-140-5p, miR-140-5p and target genes, genes and genes (TF→miRNA, miRNA→gene and gene→gene), we constructed a comprehensive TF–miR-140-5p–mRNA regulatory network using Gephi software (release 0.8.1–β, http://gephi.github.io/).

Screening target genes and signaling pathways inhibited by miR-140-5p in published studies

To obtain downstream target genes and signaling pathways modulated by miR-140-5p in published studies, a comprehensive electronic search of Web of Science and PubMed databases was performed until April 20, 2017. The keyword ‘miR-140-5p’ in the titles or abstracts was used, and then, studies exploring the targets of miR-140-5p were collected.

Results

Mature sequences of miR-140-5p in various species

Mature sequences of miR-140-5p in various species were obtained in the miRBase database. The pre-miR-140-5p was located at position 6993081–69933180 of chromosome 16, and the gene ID of human miR-140-5p was MIMAT0000431. As shown in Table 1, mature sequences of miR-140-5p were highly conserved in various species and human miR-140-5p was chosen for further analyses.
Prediction of target genes for miR-140-5p

As shown in Fig. 1, the number of predicted target genes of miR-140-5p in miRanda, TargetScan, RNAhybrid, and miRDB databases was 2370, 428, 1017, and 262, respectively. There were 482 target genes supported by at least two databases, 123 target genes predicted by at least three databases and five target genes supported by all four databases. The target genes of miR-140-5p predicted by at least three databases are listed in Table 2 and were used for further analyses.

GO enrichment analysis for predicted target genes of miR-140-5p

GO enrichment analysis was conducted for the target genes of miR-140-5p predicted by at least three databases. As shown in Table 3, the target genes of miR-140-5p were mainly located in basement membrane (P < 0.05) and participated in the molecular functions of protein binding, activating transcription factor binding, ion binding, lipid binding, and so on (P < 0.05). In addition, the target genes of miR-140-5p were involved in various biological processes, including biological regulation, metabolic process, cell communication, signal transduction, response to chemical stimulus, stem cell proliferation, cell surface receptor signaling pathway (P < 0.05). Fig. 2 presents the number of target genes corresponding to each GO term.

KEGG pathway analysis for predicted target genes of miR-140-5p

Enriched signaling pathways for the target genes of miR-140-5p identified by KEGG pathway analysis were ranked according to the P-values. As shown in Table 4, the top rankings were related to Notch, cancer-associated pathway, TGF-beta, PI3K/Akt, HTLV infection, Hippo, HIF-1, alcoholism signaling pathways, and so on (P < 0.05); among them, Notch, TGF-beta, PI3K/Akt, and Hippo signaling pathways were well known to be associated with the pathogenesis of PAH. Fig. 3 presents the rich factor, Q value, and gene number corresponding to each pathway term.
### Table 2. The target genes of miR-140-5p predicted by at least three databases.

| ABCA1  | ACSL6 | ADAM10 | ADAMTS5 | ADCY6 | ANKY1 |
|--------|-------|--------|---------|-------|--------|
| ANKIB1 | AP2B1 | BACH1  | BAZ2B   | BCL9  | BMP2   |
| C1R    | CADM3 | CAND1  | CAPN1   | CCNL1 | CELF1  |
| CORO2A | CREB  | CTCF   | CYTH2   | DNM3  | DOK4   |
| DPP10  | DPYSL2| EGR2   | EIF4G2  | ELAVL2| ENTPD5 |
| EPB41L2| ERC2  | FAM17B | FBN1    | FCHO2 | FES    |
| FGF9   | FLRT2 | FOXP2  | FYN1    | GNAS  | GIT1   |
| HAND2  | HDAC4 | HDAC7  | HGFGRP3 | HRNPH3| HS2ST1 |
| HSPA13 | IGF3  | IPI7   | JAG1    | KAT2B | KBTBD2 |
| KIF1B  | KFL6  | KLF9   | KLK10   | LAMC1 | LHFPL2 |
| LMNB1  | LPHN2 | LRAT   | LRP4    | LSM14B| LYSMD3 |
| MARK1  | MED13 | MMD    | MYCIP2  | MYO10 | NAA20  |
| NAAALD1| NCKAP1| NCOA1  | NCSTN   | NFE2L2| NLK    |
| NPL    | NUCKS1| OBPL6  | PPI1CC  | PAFAH1B2| PDGFR |
| PPTC7  | PEDE7A| PPP1R12A| PALM2-AKAP2| RBM39  | RXF7   |
| RNF19A | RALA  | RAB10  | SEPT2   | STRAD | SY1    |
| SLAIN1 | SAMD4 | SMOC2  | SNX2    | SRCAP | SHROOM3|
| SIAH1  | SLC30A5| SLC38A2| TTYH3  | TLR4  | TTK    |
| TJP1   | TSSK2 | TSPAN12| TSC2D2 | TTYH2 | TGFR1 |
| UBR5   | UBR5  | VEGF1  | VEGFA   | WNT1  | WDFY3  |
| YOD1   | ZBTB10| ZNF800 |         |       |        |

### Table 3. Gene ontology (GO) analysis for predicted target genes of miR-140-5p

| ID      | Term                                | P-value   | Genes annotated to the term |
|---------|-------------------------------------|-----------|-----------------------------|
| GO:0050794 | Regulation of cellular process       | 5.39E-06  | VEGFA|FGF9|PPP1CC|Pin1|HDAC7|PDGFR|TGFR1|ADAM10… |
| GO:0050789 | Regulation of biological process     | 9.05E-06  | FGF9|BM2|LAMC1|NUMBL|PDGFR|PPP1CC|ADAM10|TLR4|TGFB1… |
| GO:0007154 | Cell communication                   | 5.69E-05  | WNT1|PPP1CC|PDGFR|TLR4|ADAM10|BM2|TGFR1… |
| GO:0023052 | Signaling                            | 6.14E-05  | PDGFR|PPP1CC|FGF9|WNT1|TGFR1|BM2|ADAM10|JAG1|TLR4… |
| GO:0044763 | Single-organismal cellular process   | 8.73E-05  | VEGFA|FGF9|LAMC1|BM2|TLR4|WNT1|TGFR1|PDGFR|PPP1CC… |
| GO:0065007 | Biological regulation                | 9.89E-05  | VEGFA|BM2|TLR4|CREB|PPP1CC|PDGFR|ADAM10|TGFR1… |
| GO:0007165 | Signal transduction                  | 0.00011   | PPP1CC|PDGFR|WNT1|TGFR1|FGF9|VEGFA|NCSTN|TLR4|ADAM10… |
| GO:0042221 | Response to chemical stimulus        | 0.00048   | NUMBL|PPP1CC|PDGFR|VEGFA|LAMC1|TGFR1|FGF9|BM2|ADAM10|TLR4… |
| GO:0072089 | Stem cell proliferation              | 0.00087   | ACSL6|NUMBL|RAB10|HAND2|WNT1|BM2… |
| GO:0007166 | Cell surface receptor signaling pathway | 0.00370  | TLR4|WNT1|BM2|ADAM10|NCSTN|JAG1|PPP1CC|PDGFR|FGF9… |
| GO:0050896 | Response to stimulus                | 0.01555   | PPP1CC|PDGFR|WNT1|CREB|TGFR1|VEGFA|FGF9|BM2|ADAM10|TLR4… |
| GO:0019538 | Protein metabolic process           | 0.02054   | CREB|PPP1CC|PDGFR|NUMBL|TLR4|ADAM10|BM2|KAT2B|NCSTN|TGFR1… |
| GO:0006464 | Cellular protein modification process | 0.03073  | HDAC4|CREB|ADAM10|TLR4|TGFR1|PPP1CC|PDGFR… |

### Molecular functions

| GO:0005515 | Protein binding                       | 2.53E-07  | TLR4|ADAM10|PDGFR|WNT1|HDAC7|VEGFA|CREB|PPP1CC|TGFR1|FGF9… |
| GO:0005488 | Binding                               | 0.00048   | HDAC7|JAG1|MNBL|PDGFR|ADAM10|TLR4|FGF9|KAT2B|TGFR1… |
| GO:0033613 | Activating transcription factor binding | 0.00320  | EGR2|NF2L2|HDAC4|ADAM10|HAND2… |
| GO:0043167 | Ion binding                           | 0.00724   | VEGFA|PPP1CC|ADAM10|PDGFR|TGFR1|ADAM4|FGF9|HDAC7… |
| GO:0008289 | Lipid binding                         | 0.04471   | LAMC1|OSBPL6|FES|DNM3|MYO10|TLR4… |

### Cellular components

| GO:0005604 | Basement membrane                     | 0.04119   | FGF9|PDGFR|TLR4|VEGFA|SMOC2… |
Prediction of upstream TFs for miR-140-5p and construction of TF–miR-140-5p–mRNA network

The number of predicted TFs for miR-140-5p with P-value <0.0001 was 393. To reduce false-positive results, TFs with a quality score (Q-score) less than 10 were filtered. As shown in Table 5, the remaining TFs, including PAX5, FOXI1, IRF1, FOXL1, RUNX2, were chosen for further analyses. Finally, by merging the regulatory relationships between TFs and miR-140-5p, miR-140-5p and target genes, as well as genes and genes, we built a comprehensive TF–miR-140-5p–mRNA regulatory network, as shown in Fig. 4.

Screening target genes and signaling pathways modulated by miR-140-5p in published studies

A comprehensive electronic search of Web of Science and PubMed databases was performed until April 20, 2017, to obtain target genes and signaling pathways modulated by miR-140-5p in published studies.
Table 4. Kyoto Encyclopedia of Genes and Genome (KEGG) pathway analysis for predicted target genes of miR-140-5p.

| Term                                      | ID        | Sample number | Background number | P-value   | Genes                                                                 |
|-------------------------------------------|-----------|---------------|-------------------|-----------|-----------------------------------------------------------------------|
| Notch signaling pathway                   | hsa04330  | 4             | 52                | 0.006408  | JAG1|ADAM10|KAT2B|NCSTN                         |
| Pathways in cancer                        | hsa05200  | 9             | 337               | 0.016384  | FGF9|TGFBR1|VEGFA|SLC2A1|WNT1|BMP2|PDGFRA|LAMC1                       |
| Endocrine and other factor-regulated calcium reabsorption | hsa04961  | 3             | 48                | 0.022347  | AP2B1|ADCY6|DNM3                         |
| HTLV-I infection                          | hsa05166  | 7             | 268               | 0.031935  | TGFBR1|KAT2B|SLC2A1|EGR2|WNT1|PDGFRA|ADCY6|PP1CC                         |
| Regulation of actin cytoskeleton          | hsa04810  | 6             | 221               | 0.031935  | PPP1R12A|NCAP1|FGF9|GIT1|PDGFRA|PPP1CC                         |
| Pancreatic cancer                         | hsa05212  | 3             | 66                | 0.031935  | RALA|TGFBR1|VEGFA                         |
| Epithelial cell signaling in Helicobacter pylori infection | hsa05120  | 3             | 66                | 0.031935  | TJP1|GIT1|ADAM10                         |
| Proteoglycans in cancer                   | hsa05205  | 6             | 231               | 0.033735  | PPP1R12A|FGF9|VEGFA|WNT1|TLR4|PP1CC                         |
| Adherence junction                        | hsa04520  | 3             | 74                | 0.037848  | NLK|TJP1|TGFBR1                         |
| Alcoholism                                | hsa05034  | 5             | 183               | 0.038881  | HDAC7|HDAC4|CREB3L1|GNG5|PP1CC                         |
| PI3K-Akt signaling pathway                | hsa04151  | 7             | 358               | 0.045545  | FGF9|VEGFA|PDGFRA|LAMC1|TLR4|CREB|GNG5                         |
| Focal adhesion                            | hsa04510  | 5             | 214               | 0.045545  | PPP1R12A|VEGFA|PDGFRA|LAMC1|PP1CC                         |
| Endocytosis                               | hsa04144  | 5             | 212               | 0.045545  | AP2B1|TGFBR1|GIT1|PDGFRA|DNM3                         |
| Viral carcinogenesis                       | hsa05203  | 5             | 213               | 0.045545  | HDAC7|HDAC4|KAT2B|EGR2|CREB3L1                         |
| Hepatitis B                               | hsa05161  | 4             | 151               | 0.045545  | TGFBR1|EGR2|TLR4|CREB3L1                         |
| Insulin secretion                         | hsa04911  | 3             | 92                | 0.045545  | SLCA1|CREB3L1|ADCY6                         |
| GABAergic synapse                         | hsa04727  | 3             | 89                | 0.045545  | SLCA2|CREB3L1|ADCY6                         |
| TGF-beta signaling pathway                | hsa04350  | 3             | 83                | 0.045545  | TGFBR1|SMAD4|BMP2                         |
| Gap junction                              | hsa04540  | 3             | 96                | 0.045545  | TJP1|PDGFRA|ADCY6                         |
| Hippo signaling pathway                   | hsa04390  | 4             | 156               | 0.045655  | TGFBR1|WNT1|BMP2|PP1CC                         |

Fig. 3. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis for predicted target genes of miR-140-5p.
Finally, a total of 26 papers including 23 target genes and seven signaling pathways inhibited by miR-140-5p were obtained; most of them focus on the functions of miR-140-5p suppressing tumor growth, migration, and invasion in various tumor tissues and cells. Two recent studies have found that SMURF1 and Dumt1 are direct target genes of miR-140-5p in pulmonary arterial smooth muscle cells (PASMCs) and are involved in the pathogenesis of PAH. The details are shown in Table 6.

### Discussion

Pulmonary arterial hypertension is a chronic life-threatening condition requiring long-term management [13], and its available therapies are limited [6]. There is a clear and urgent need for new therapeutic options based on deeply exploring the pathogenesis of PAH. Previous studies have indicated that miR-140-5p is dramatically downregulated, which in turn causes the development of a variety of cancers by the loss of suppressing tumor cell migration and growth [14–17]. miR-140-5p has been recently found to be reduced in both PAH patients and MCT-induced PAH models in rat [11,12]. However, the downstream targets regulated by miR-140-5p contributing to the development of PAH remain largely unknown.

In this study, we found that the target genes of miR-140-5p were enriched in many biological processes, such as biological regulation, metabolic process, cell communication, signal transduction, response to chemical stimulus, stem cell proliferation, cell surface receptor signaling pathway. In KEGG pathway analysis, the target genes of miR-140-5p were mainly located in Notch, TGF-beta, PI3K/Akt, and Hippo signaling pathways. According to the TF–miRNA–mRNA network, the important genes potentially regulated by miR-140-5p included PPI, TGF-betaR1, smad4, JAG1, ADAM10, FGF9, PDGFRA, VEGFA, TLR4, LAMC1, CREB, and the upstream TFs, which might regulate miR-140-5p expression including TAX5, FOXI, IRF1, GATA6, RUNX2. After thoroughly reviewing published literature, we found that 23 target genes and seven signaling pathways were truly inhibited by miR-140-5p in various tissues or cells; most of these downstream targets were in accordance with our present prediction.

Several studies have shown that activation of Notch3 pathway is involved in the pathogenesis of PAH [18,19]. We have previously shown that

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### Table 5. Prediction of transcription factors and binding sites of miR-140-5p.

| Model ID  | Model name | Hit position | Strand | Score  | Predicted site sequence |
|-----------|------------|--------------|--------|--------|-------------------------|
| MA0014.2  | PAX5       | 95           |        | 10.5663| gtctcactctgtgccccat     |
| MA0014.2  | PAX5       | 3874         |        | 11.6915| gtcttgtctgtgccccag      |
| MA0025.1  | NFIL3      | 722          |        | 10.0393| TCTCTACATAA             |
| MA0035.3  | Gata1      | 3391         |        | 10.0718| agacataaaaa             |
| MA0036.2  | GATA2      | 3391         |        | 10.4087| agacataaaaaattt        |
| MA0041.1  | Foxd3      | 4529         | +      | 10.4011| ttgttgtggtt             |
| MA0041.1  | FOXI1      | 984          |        | 11.5926| GGATGTTGTTT             |
| MA0042.1  | FOXI1      | 4529         | +      | 10.3990| ttgttgtggtt             |
| MA0046.1  | HNF1A      | 4949         | +      | 10.3282| aagtaatattta            |
| MA0050.2  | IRF1       | 3825         | +      | 11.0065| ttttttttttctttttttttt   |
| MA0050.2  | IRF1       | 3840         | +      | 12.4803| ttttttttttttttttttttttt |
| MA0050.2  | IRF1       | 3844         | +      | 10.0776| ttttttttttttttttttttttt |
| MA0062.2  | GABPA      | 1506         | +      | 10.0387| ccggaagctg             |
| MA0073.1  | RREB1      | 1164         |        | 10.9028| TTTTGGTTGTTGTTTGTGTTT  |
| MA0073.1  | RREB1      | 3734         | +      | 10.2056| caaacaacaacaacaacaaca  |
| MA0471.1  | E2F6       | 143          |        | 10.6410| ttcttcgccct             |
| MA0477.1  | FOSL1      | 4238         |        | 11.2229| cctgtagcacc             |
| MA0478.1  | FOSL2      | 4239         |        | 10.3145| ctgtagcacc              |
| MA0481.1  | FOXP1      | 3756         | +      | 10.2195| aaaaaaaaaaaaacaa        |
| MA0481.1  | FOXP1      | 4018         | +      | 10.3465| ttgtgtgtgtgtggtgtgtggtg |
| MA0490.1  | JUNB       | 4239         | +      | 10.6046| ctgtagcacc              |
| MA0491.1  | JUND       | 2362         | +      | 10.0256| GAAATGATACACA           |
| MA0493.1  | Klf1       | 4812         | +      | 10.548 | caacacacacaci          |
| MA0511.1  | RUNX2      | 3813         | +      | 11.453 | tttgtgtggtgtgtgtgtggtg |
| MA0515.1  | Sox6       | 3772         |        | 10.2529| gacacacacag            |
| MA0595.1  | SREBF1     | 2000         |        | 10.1772| gigaagtgggtg           |
activation of Notch3 promotes PASMC proliferation and inhibition of Notch3 pathway prevents monocrotaline-induced development of PAH in rat [20,21]. JAG1 and ADAM10 are indispensable components of Notch signaling pathway, which were predicted as downstream targets of miR-140-5p in our analysis, suggesting that lack of miR-140-5p might promote the development of PAH by upregulation of JAG1 and ADAM10 genes and therefore activation of Notch3 cascade. In addition, activation of TGF-beta1/Smad4 signaling promotes a proliferative PASMC phenotype and induces PAH in rat [22,23]. We found that TGF-betaR1 and smad4 were possible downstream targets of miR-140-5p, reduction in miR-140-5p in PAH might stimulate TGF-beta1/Smad4 pathway by upregulating TGF-betaR1 and smad4. Previous studies have demonstrated that PDGF, TLR4, VEGFA, and FGF contribute to the pathogenesis of PAH via activating various signaling pathways, especially PI3K/Akt cascade [24–28]. CREB, an important transcription factor lying downstream of PI3K/Akt pathway, mediates the partial functions of PI3K/Akt [29]. In our analysis, PDGF, TLR4, VEGFA, FGF, and CREB were positively predicted as downstream targets of miR-140-5p, implying that miR-140-5p negatively regulates the functions of PI3K/Akt cascade by targeting FGF9, PDGFRA, VEGFA, TLR4, or CREB gene. Recent studies have also shown that Hippo signaling is associated with the development of PAH, which can be activated by PPI [30,31]. Our present results suggested that PPI was a direct target gene of miR-140-5p and might mediate miR-140-5p regulation of Hippo signaling.

Our predicted network provided potential target genes and relevant signaling pathways that might be modulated by miR-140-5p contribution to the
development of PAH. Several targets and pathways predicted in our analysis, such as TGF-betaR1, ADAM10, FGF9, PDGFRα, VEGFA and Notch, PI3K/Akt, TGF-beta cascades, have been demonstrated to mediate the effects of miR-140-5p on antiproliferation and prodifferentiation in several cell types in published studies [16,17,32,33]. While the other targets predicted in our study, including PPI, smad4, JAG1, LAMC1, TLR4, and CREB as well as Hippo signaling pathway, have not been confirmed in
the published literature, they still need further verification in vivo and in vitro.

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Author contributions
ML and FL designed the study; WS, YW, LC, and QW analyzed and interpreted the data; WF, XY, QZ, and JW organized the results; FL wrote the manuscript.

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