Non-Coding RNA Networks in Pulmonary Hypertension

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Non-coding RNAs (ncRNAs) are involved in various cellular processes. There are several ncRNA classes, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). The detailed roles of these molecules in pulmonary hypertension (PH) remain unclear. We systematically collected and reviewed reports describing the functions of ncRNAs (miRNAs, lncRNAs, and circRNAs) in PH through database retrieval and manual literature reading. The characteristics of identified articles, especially the experimental methods, were carefully reviewed. Furthermore, regulatory networks were constructed using ncRNAs and their interacting RNAs or genes. These data were extracted from studies on pulmonary arterial smooth muscle cells, pulmonary artery endothelial cells, and pulmonary artery fibroblasts. We included 14 lncRNAs, 1 circRNA, 74 miRNAs, and 110 mRNAs in the constructed networks. Using these networks, herein, we describe the current knowledge on the role of ncRNAs in PH. Moreover, these networks actively provide an improved understanding of the roles of ncRNAs in PH. The results of this study are crucial for the clinical application of ncRNAs.

Keywords: pulmonary hypertension, long non-coding RNA, circular RNA, microRNA, network

1 INTRODUCTION

Pulmonary hypertension (PH) is a serious disease characterized by progressively increased pulmonary vascular resistance and pulmonary artery pressure; the diagnostic criterion is mean pulmonary artery pressure ≥25 mmHg (Galiè et al., 2016; Weber et al., 2018). The increased pulmonary artery pressure in PH results from changes in the structure and function of the vessel wall, which is induced by abnormal pulmonary cell proliferation, apoptosis, and migration (Bourgeois et al., 2018a). Patients with PH may experience dyspnea, fatigue, syncope, chest pain, and/or edema of the legs and ankles. The causes of PH can be broadly classified as primary and secondary causes. To date, ion channels, vasoactive substances, immune factors, and genetic factors are known to be involved in the pathogenesis of PH (Chelladurai et al., 2016; Veith et al., 2016; Bourgeois et al., 2018b).

Recently, many non-coding RNAs (ncRNAs) have been recognized as important regulators in the development of PH. Most human genes (>95%) do not produce proteins but ncRNA molecules. Among them, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) are the most widely studied. MiRNAs are small ncRNAs containing 21–22 nucleotides, which post-transcriptionally regulate gene expression (Wakita and Yokoyama, 2014). LncRNAs, which have more than 200 nucleotides, are transcribed from intergenic or intragenic regions. They can bind to proteins, RNA, or DNA to execute regulatory roles (Botti et al., 2017). CircRNAs are a novel class of ncRNAs with a closed loop structure, making them highly stable and capable of interacting with proteins or RNA (Di et al., 2019). NcRNAs have been identified...
to regulate multiple steps of gene expression. However, because of the large quantity and diverse mechanisms, it is difficult to comprehensively understand the roles of ncRNAs.

NcRNA-based therapeutics have emerged for several diseases, including PH. An effective ncRNA-based strategy demands a thorough understanding of the diverse and context-dependent regulatory relationships of ncRNAs. The regulation of gene expression by ncRNAs is frequently cell specific, suggesting that not only expression level, but also activity or bioavailability contribute to the biofunction of ncRNAs (Correia de Sousa et al., 2019). Thus, in this article, we reviewed other public databases, including the Human microRNA Disease Database v3.2, miRWalk 2.0, and LncRNADisease v2.0, to identify validated functional ncRNAs in PH. Studies were selected when the following criteria were met: 1) the study reported pathogenic roles of miRNAs, lncRNAs, and/or circRNAs in PH; 2) mechanistic studies were performed in pulmonary arterial smooth muscle cells (PASMCs), pulmonary artery endothelial cells (PAECs), and/or pulmonary artery fibroblasts (PAFs); and 3) the relationships between ncRNAs and their interacting RNAs or genes were experimentally identified via luciferase reporter assay, western blot, and/or qPCR. Using these criteria returned 140 qualified articles (Figure 1).

2 SCREENING OF ARTICLES
2.1 Criteria for Study Selection
A literature search was performed in PubMed with the query listed in Table 1; we identified 602 articles. In addition, we also reviewed other public databases, including the Human microRNA Disease Database v3.2, miRWalk 2.0, and LncRNADisease v2.0, to identify validated functional ncRNAs in PH. Studies were selected when the following criteria were met: 1) the study reported pathogenic roles of miRNAs, lncRNAs, and/or circRNAs in PH; 2) mechanistic studies were performed in pulmonary arterial smooth muscle cells (PASMCs), pulmonary artery endothelial cells (PAECs), and/or pulmonary artery fibroblasts (PAFs); and 3) the relationships between ncRNAs and their interacting RNAs or genes were experimentally identified via luciferase reporter assay, western blot, and/or qPCR. Using these criteria returned 140 qualified articles (Figure 1).

2.2 General Characteristics of Qualified Articles
When sorted by publication date, we found that the number of eligible articles continuously increased year by year (Figure 2A). The impact factors (IF) of the articles ranged from 0 to 36.13; articles with 3 ≤ IF < 5 accounted for the highest proportion (Figure 2B). Of the 140 qualified articles, 32.14% were studies using human tissues or cells. In studies using experimental animals, rats were the most commonly used, accounting for 26.43% of the total studies (Figure 2C). Moreover, when classified by cell type, 78.42, 15.83, 1.44, and 4.32% of studies were performed in PASMCs, PAECs, PAFs, and both PASMCs and PAECs, respectively (Figure 2D).

3 NON-CODING RNA NETWORKS FOR PULMONARY HYPERTENSION
3.1 Construction of Non-coding RNA Regulatory Networks
Regulatory networks were constructed using ncRNAs and their interacting RNAs or genes in PASMCs, PAECs, and PAFs. Given ncRNA conservation among species, only human ncRNAs or ncRNAs that were conserved between human and experimental animals were included. If there were contradictory results, the results from higher-impact articles were selected. In addition, some crucial regulatory relationships between protein-coding genes and validated transcription factor–miRNA interactions from TransmiR v2.0 were also described in the networks to present an in-depth explanation on the roles of ncRNAs in PH. The nodes represented interacting molecules, and the edges represented the regulatory connections. Each edge indicated a publication supporting the connection. Square and
circular nodes represented ncRNAs and coding RNAs or genes, respectively. Node color was based on the type of molecule (LncRNAs and circRNAs are orange, miRNAs are blue, and coding RNAs or genes are empty). Node sizes represented their degrees (number of edges that directly link to the node). Edges represented the regulatory connections: red edges depicted links indicating repressive action (semicircular arrow heads), and black edges indicated activation (traditional arrow heads). The nodes in this network were involved in cell proliferation, apoptosis, migration, metabolism, endothelial-mesenchymal transition, and extracellular matrix remodeling. The steps used in our approach are shown in Figure 1.

3.2 General Characteristics of the Constructed Networks

In total, 140 articles describing 14 LncRNAs, 1 circRNA, 74 miRNAs, and 110 mRNAs, were included in our networks. Considering the unique biological characteristics of different cell types, we constructed networks according to cell type. The
network of PASMCs contained 13 lncRNAs, 1 circRNA, 69 miRNAs, and 96 mRNAs. The network of PAECs contained 1 lncRNA, 25 miRNAs, and 29 mRNAs. The network of PAFs contained 6 miRNAs and four mRNAs. The networks are shown in Figures 3–5. Detailed network compositions are listed in Table 2.

3.3 Functional Enrichment Analysis
We performed gene ontology (GO) biological process term analyses and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses using the database for Annotation, Visualization, and Integration Discovery (DAVID). The calculation process was dependent on a hypergeometric test, using a statistical significance threshold of \( p < 0.05 \) with a false discovery rate (FDR) correction. These analyses provided a general overview of the biological roles of the included ncRNAs. In addition, we performed cell type-specific functional enrichment analyses. However, owing to the lack of sufficient molecules, the enrichment analysis in PAFs could not be conducted. GO analysis and KEGG pathway enrichment in PASMCs and PAECs revealed several PH-associated terms, such as positive regulation of smooth muscle cell proliferation, positive regulation of endothelial cell proliferation, HIF-1 signaling pathway, and MAPK signaling pathway. The top 10 enriched GO biological process terms and KEGG pathways are shown in Figure 6.
3.4 Key Non-coding RNA Subnetworks

We built three networks according to the cell types. Here, we discuss several important subnetworks, along with their components and interactions, to improve understanding of the roles of ncRNAs in PH. Subnetworks with more than five nodes were regarded as key subnetworks.

3.4.1 The Hsa_circ_0016070/miR-942-5p/CCND1 Subnetwork

CircRNAs are associated with various cardiovascular diseases. Hsa_circ_0016070 was the only circRNA included in our networks. This circRNA is located at chr1: 203595914-203702528, strand: +, promotes cell proliferation by mediating cell cycle progression, and is increased in PH patients (Zhou et al., 2019). CCND1 is an important regulator of the cell cycle. It interacts with cyclin-dependent kinase 4 (CDK4) to form the cyclin D1–CDK4 complex, which then inactivates retinoblastoma (Rb) protein and induces G0 progression to S phase (Matsushime et al., 1991). The subnetwork showed that hsa_circ_0016070 overexpression induced CCND1 expression by buffering miR-942-5p (Zhou et al., 2019). In addition, according to our network, CCND1 could induce the expression of miR-17-5p, miR-19a-3p, and miR-20a-5p, subsequently regulating the biological activities of PASMCs (Figure 7A). Given the considerable number of identified circRNAs, there should be other PH-related circRNAs. A microarray expression profile in thromboembolic pulmonary hypertension patients indicated that hsa_circ_0002062 and hsa_circ_0022342 might be the key circRNAs for the development of chronic thromboembolic pulmonary hypertension (Miao et al., 2017). However, this finding has not been verified by more reliable experimental methods.

3.4.2 The TUG1/MEG3/miR-328-3p/miR-193-3p Subnetwork

Regulatory relationships are indicated by the connection lines in the subnetwork. According to this subnetwork (Figure 7B), both...
TUG1 and MEG3 can function as competing endogenous RNAs (ceRNAs) that sequester miR-328-3p. In the original studies, the TUG1/miR-328-3p and MEG3/miR-328-3p axes were identified (Wang D et al., 2019; Xing X.-Q et al., 2019). IGF1 is reported to inhibit PASMCs apoptosis and activate elastin in PASMCs. Thus, upregulating IGF1 via the TUG1/miR-328-3p and MEG3/miR-328-3p axes can induce PH by amplifying the pathogenic role of IGF1 (Wang S et al., 2019; Xing Y et al., 2019). Calcium voltage-gated channel subunit alpha1 C (CaV1.2), which contributes to vasoconstriction, is also a target gene of miR-328-3p in PASMCs (Guo et al., 2012), indicating that the TUG1/miR-328-3p and MEG3/miR-328-3p axes are involved in regulating pulmonary artery contraction and dilation. In addition, miR-328-3p can inhibit PASMC proliferation by targeting PIM-1 (Qian et al., 2020). P27 and TIMP3 are two additional target genes of miR-222-3p (Xu et al., 2017). P27, a member of the Cip/Kip family of cyclin-dependent kinase inhibitors, negatively regulates cell proliferation (Toyoshima and Hunter, 1994). Meanwhile, TIMP3 is a member of the TIMP family, which regulates cell proliferation, apoptosis, and migration via both MMP-dependent or MMP-independent pathways (Zhou et al., 2015). The present subnetwork links CASC2 to P27 and TIMP3 via miR-222-3p, further elaborating the mechanisms of PH (Figure 7C).

UCA1 is the other lncRNA in this subnetwork and is highly expressed in hypoxia-induced PASMCs. Studies indicate that UCA1 does not interact with miR-222-3p, but directly inhibits ING5 by competing with ING5 mRNA for hnRNP I, which binds to ING5 mRNA and enhances its translation. Thus, UCA1 overexpression results in the downregulation of ING5 mRNA expression (Zhu T.-T, et al., 2019). The same regulatory pattern has been found between UCA1 and P27 in breast tumor studies (Huang et al., 2014). This interaction may also work in PH and partly contributes to P27 downregulation (Figure 7C).

3.4.4 The MALAT1/miR-124-3p Subnetwork
LncRNA MALAT1, located at 11q13, is an 8.5-kb molecule that was identified by Ji et al. in a cancer study (Ji et al., 2003). Emerging evidence indicates that MALAT1 plays important roles in various diseases, including PH. Wang et al. reported that MALAT1 is highly expressed in pulmonary artery tissues and PASMCs from patients with PH. MALAT1 controls PASMC proliferation and migration by binding to miR-124-3p, which directly targets KLF5 (Wang D et al., 2019). Kang et al. showed that miR-124-3p also targets three regulators of the NFAT pathway, including NFATc1, CAMTA1, and PTBP1 (Kang B.-Y et al., 2013). The downregulation of miR-124-3p induces PASMC proliferation and reverses the differentiated PASMC phenotype by activating the NFAT pathway. In addition to its role in PASMCs, miR-124-3p also regulates the biological behaviors of PAH endothelial cells (PAH ECs) and PAFs. Studies have confirmed the role of the miR-124-3p/PTBPI axis in PAH ECs and PAFs (Caruso et al., 2017; Wang et al., 2014; Zhang H et al., 2017). Downregulating miR-124-3p activates PTBPI expression, which promotes aerobic glycolysis by increasing the PKM2/PKM1 ratio, subsequently inducing PAH EC and PAF proliferation (Anastasiou et al., 2012). Li et al. reported another target of miR-124-3p, GRB2, which enhanced the proliferation of multiple human cells (Li L et al., 2017; Figure 7D).

3.4.5 Subnetworks of the miR-130/301 Family
There are complicated relationships between the miR-130/301 family and other functional molecules associated with the pathogenesis of PH. In the present study, we found that
### TABLE 2 | List of network interactions.

| Upstream molecule | Downstream molecule | Interaction type | PMID            | Reference                  |
|-------------------|--------------------|------------------|-----------------|----------------------------|
| ALOX12            | RXR-a              | pos              | 24963038        | Sharma et al. (2014)       |
| ALOX15            | RXR-a              | pos              | 24963038        | Sharma et al. (2014)       |
| ALOX5             | RXR-a              | pos              | 24963038        | Sharma et al. (2014)       |
| APLN              | miR-424-5p         | pos              | 23263626        | Kim et al. (2013)          |
| APLN              | miR-503-5p         | pos              | 23263626        | Kim et al. (2013)          |
| CASC2             | miR-222-3p         | neg              | 32206065        | Han et al. (2020)          |
| CCN1              | miR-17-5p          | neg              | 18695042        | Yu et al. (2008)           |
| CCN1              | miR-19a-3p         | pos              | 28090171        | Inoue and Fry. (2015)      |
| CCN1              | miR-20a-5p         | pos              | 28090171        | Inoue and Fry. (2015)      |
| CPS1-T            | IL-1β              | neg              | 30982984        | Zhang et al. (2019b)       |
| CREB1             | MCU                | pos              | 27648337        | Hong et al. (2017)         |
| H19               | let-7b-5p          | neg              | 30547791        | Su et al. (2018)           |
| HDAC4             | miR-424-5p         | neg              | 29012771        | Takagi et al. (2018)       |
| HDAC4             | miR-503-5p         | neg              | 29012771        | Takagi et al. (2018)       |
| HDAC5             | miR-424-5p         | neg              | 29012771        | Takagi et al. (2018)       |
| HDAC5             | miR-503-5p         | neg              | 29012771        | Takagi et al. (2018)       |
| HIF-1α            | let-7b-3p          | pos              | 30628484        | Zhang H et al. (2019)      |
| HIF-1α            | miR-145-5p         | pos              | 25129238        | Agrawal et al. (2014)      |
| HIF-1α            | miR-191-5p         | pos              | 25119596        | Song et al. (2014)         |
| HIF-1α            | miR-195-5p         | pos              | 28862358        | Zeng et al. (2018)         |
| HIF-1α            | miR-19a-3p         | pos              | 31682384        | Zhao et al. (2019)         |
| HIF-1α            | miR-205-3p         | pos              | 23622208        | Gandelli et al. (2014)     |
| HIF-1α            | miR-210-3p         | neg              | 30547791        | Guo et al. (2014)          |
| HIF-1α            | miR-214-3p         | pos              | 24011070        | el Azouzi et al. (2013)    |
| HIF-1α            | miR-223-3p         | neg              | 29064306        | Meloche et al. (2015a)     |
| HIF-1α            | miR-27a-3p         | neg              | 2457586         | Camps et al. (2014)        |
| HIF-1α            | miR-361-5p         | pos              | 29390976        | Zhang Y et al. (2018)      |
| HOXA-AS3          | HOXA3              | pos              | 30304383        | Zhang R P et al. (2019)    |
| LncRNA-Cox2       | let-7a             | neg              | 31593832        | Zhou et al. (2019)         |
| LncRNA-Ang362     | MANTIS             | neg              | 26308365        | Cheng et al. (2020)        |
| LncRNA-Ang362     | let-7a             | neg              | 32986284        | Zhang Y et al. (2019)      |
| LncRNA-Ang362     | let-7b-3p          | neg              | 30547791        | Su et al. (2018)           |
| LncRNA-Ang362     | let-7b-5p          | neg              | 30547791        | Guo et al. (2014)          |
| LncRNA-Ang362     | let-7b-5p          | neg              | 24978844        | Guo et al. (2014)          |
| LncRNA-Ang362     | let-7b-5p          | neg              | 24978844        | Guo et al. (2014)          |
| LncRNA-Ang362     | STAT3              | neg              | 23851900        | Leisegang et al. (2017)    |
| LncRNA-Ang362     | ACE2               | neg              | 30628484        | Cheng et al. (2020)        |
| LncRNA-Ang362     | AT1R               | neg              | 23851900        | Wang et al. (2020)         |
| LncRNA-Ang362     | ET-1               | neg              | 23851900        | Wang et al. (2020)         |
| LncRNA-Ang362     | TGFBR1             | neg              | 23851900        | Wang et al. (2020)         |
| LncRNA-Ang362     | MYC                | neg              | 27889560        | Wang et al. (2020)         |
| LncRNA-Ang362     | PTBP1              | neg              | 26365914        | Wang et al. (2020)         |
| MALAT1            | miR-124-3p         | neg              | 30313741        | Wang et al. (2020)         |
| MANTIS            | BRG1               | pos              | 2.8351900       | Wang et al. (2020)         |
| MEG33             | miR-328-3p         | neg              | 31477557        | Wang et al. (2015)         |
| miR-100-5p        | MTOR               | neg              | 24978844        | Deng et al. (2016)         |
| miR-103a-3p       | HIF-1α             | neg              | 26627991        | Deng et al. (2016)         |
| miR-107-3p        | HIF-1α             | neg              | 26627991        | Deng et al. (2016)         |
| miR-107-3p        | NOR1               | neg              | 31933977        | Deng et al. (2016)         |
| miR-1181          | STAT3              | neg              | 30211651        | Deng et al. (2016)         |
| miR-124-3p        | CAMTA1             | neg              | 23853098        | Qian et al. (2018)         |
| miR-124-3p        | GRB2               | neg              | 28496318        | Li Y et al. (2017)         |
| miR-124-3p        | KLF5               | neg              | 31257328        | Wang et al. (2019)         |
| miR-124-3p        | NFATC1             | neg              | 23853098        | Wang et al. (2019)         |
| miR-124-3p        | PTBP1              | neg              | 23853098        | Wang et al. (2019)         |
| miR-124-3p        | PTBP1              | neg              | 2.8791999       | Wang et al. (2019)         |
| miR-125a-5p       | BMPR2              | neg              | 25584587        | Caruso et al. (2017)       |
| miR-125a-5p       | BMP2               | neg              | 28593577        | Ma et al. (2017)           |
| miR-125a-5p       | STAT3              | neg              | 29070287        | Cai et al. (2018)          |
| miR-1268a         | CDKN2A(P16)        | neg              | 31370272        | Lee and Kang. (2019)       |
| miR-1281          | HDAC4              | neg              | 29514810        | Li et al. (2018)           |
| miR-130a-3p       | BMPR2              | neg              | 28755990        | Li et al. (2017)           |
| miR-130a-3p       | CDK4/6A(P21)       | neg              | 25681685        | Broek et al. (2015)        |
| miR-130a-3p       | LRP8               | neg              | 26365914        | Bertero et al. (2015)      |

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| Upstream molecule | Downstream molecule | Interaction type | PMID       | Reference               |
|-------------------|---------------------|------------------|------------|-------------------------|
| miR-130a-3p       | PPARγ              | neg              | 24960162   | Bertero et al. (2014)   |
| miR-130a-3p       | PPARγ              | neg              | 26565914   | Bertero et al. (2015)   |
| miR-130b-3p       | LRP8               | neg              | 24960162   | Bertero et al. (2014)   |
| miR-130b-3p       | PPARγ              | neg              | 26565914   | Bertero et al. (2015)   |
| miR-132-3p        | PTEN               | neg              | 30896881   | Zeng et al. (2019)      |
| miR-135a-5p       | TRPC1              | neg              | 30033839   | Liu A et al. (2019)     |
| miR-138-5p        | CREB1              | neg              | 27648837   | Hong et al. (2017)      |
| miR-138-5p        | MCU                | neg              | 27648837   | Hong et al. (2017)      |
| miR-138-5p        | MST1               | neg              | 23485012   | Li et al. (2013)        |
| miR-138-5p        | TASK-1             | neg              | 29257242   | Li G et al. (2018)      |
| miR-135a-5p       | VEGF               | neg              | 31894295   | Zhang et al. (2020)     |
| miR-17-5p         | BMP2               | neg              | 19300956   | Brock et al. (2009)     |
| miR-17-5p         | CDKN1A(P21)        | neg              | 30305109   | Liu J. J et al. (2018)  |
| miR-17-5p         | MFN2               | neg              | 27640176   | Lu et al. (2016)        |
| miR-17-5p         | PAI-1              | neg              | 29644896   | Chen K.-H et al. (2018) |
| miR-17-5p         | PDLIM5             | neg              | 25647182   | Chen et al. (2015)      |
| miR-17-5p         | PHD2               | neg              | 27919930   | Chen et al. (2016)      |
| miR-17-5p         | PTEN               | neg              | 30035109   | Li G et al. (2018)      |
| miR-182-3p        | MYADM              | neg              | 32372333   | Sun et al. (2020)       |
| miR-190a-5p       | KCNQ5              | neg              | 24446351   | Li et al. (2014)        |
| miR-190a-5p       | KLF15              | neg              | 30538440   | Jiang et al. (2018)     |
| miR-191-5p        | BMP2               | neg              | 31119161   | Zhang Z et al. (2019)   |
| miR-193-3p        | ALOX12             | neg              | 24963038   | Sharma et al. (2014)    |
| miR-193-3p        | ALOX15             | neg              | 24963038   | Sharma et al. (2014)    |
| miR-193-3p        | ALOX5              | neg              | 24963038   | Sharma et al. (2014)    |
| miR-193-3p        | IGFL1              | neg              | 24963038   | Sharma et al. (2014)    |
| miR-195-5p        | SMAD7              | neg              | 28862358   | Zhang et al. (2018)     |
| miR-199a-5p       | SMAD3              | neg              | 27038547   | Liu H et al. (2016)     |
| miR-199b-5p       | GSK3B              | neg              | 27188753   | Wu et al. (2016)        |
| miR-19a-3p        | PAI-1              | neg              | 29644896   | Chen T et al. (2018)    |
| miR-19a-3p        | PTEN               | neg              | 31682884   | Zhao et al. (2019)      |
| miR-19b-3p        | PAI-1              | neg              | 29644896   | Chen K.-H et al. (2018) |
| miR-200c-3p       | MAP2               | neg              | 29044995   | Yuan et al. (2017)      |
| miR-200c-3p       | ZEB1               | neg              | 29044995   | Yuan et al. (2017)      |
| miR-203a-3p       | FGF2               | neg              | 30575929   | Wang et al. (2019)      |
| miR-204-5p        | ATG7               | neg              | 31542480   | Liu H.-N et al. (2019)  |
| miR-204-5p        | BRD4               | neg              | 26224795   | Meloche et al. (2015a)  |
| miR-204-5p        | FOXM1              | neg              | 29290032   | Bourgeois et al. (2018b) |
| miR-204-5p        | RUNX2              | neg              | 27149112   | Ruffenach et al. (2018) |
| miR-204-5p        | SHP2               | neg              | 21321078   | Courboulin et al. (2011) |
| miR-204-5p        | TGFBR2             | neg              | 29196166   | Yu et al. (2018)        |
| miR-205-5p        | MICAL2             | neg              | 30653343   | Tao et al. (2019)       |
| miR-206           | Notch3             | neg              | 23071643   | Jialal et al. (2012)    |
| miR-206           | HIF-1α             | neg              | 23628900   | Yue et al. (2013)       |
| miR-20a-5p        | BMP2               | neg              | 19300956   | Brock et al. (2009)     |
| miR-20a-5p        | PAI-1              | neg              | 29644896   | Chen T et al. (2018)    |
| miR-20a-5p        | PDLIM5             | neg              | 25647182   | Chen et al. (2015)      |
| miR-20a-5p        | PHD2               | neg              | 27919930   | Chen et al. (2016)      |
| miR-210-3p        | E2F3               | neg              | 22886504   | Gou et al. (2012)       |
| miR-210-3p        | ISCU1/2            | neg              | 25825391   | White et al. (2015)     |
| miR-210-3p        | MKP-1              | neg              | 25044272   | Jin et al. (2015)       |
| miR-214-3p        | ARHGEF12           | neg              | 31373336   | Xing Y et al. (2019)    |
| miR-214-3p        | CCNL2              | neg              | 27381447   | Liu Y et al. (2016)     |
| miR-214-3p        | LMOD1              | neg              | 27144530   | Sahoo et al. (2016)     |

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TABLE 2 | (Continued) List of network interactions.

| Upstream molecule | Downstream molecule | Interaction type | PMID | Reference |
|-------------------|---------------------|-----------------|------|-----------|
| miR-214-3p        | MEF2C               | neg             | 27144530 | Sahoo et al. (2016) |
| miR-214-3p        | PTEN                | neg             | 28684904 | Liu et al. (2017) |
| miR-21-5p         | DDAH1               | neg             | 24895913 | Iannone et al. (2014) |
| miR-21-5p         | PDCD4               | neg             | 28522368 | Green et al. (2017) |
| miR-21-5p         | PTEN                | neg             | 26526966 | Green et al. (2015) |
| miR-21-5p         | RHOB                | neg             | 22571328 | Parikh et al. (2012) |
| miR-221-3p        | AXIN2               | neg             | 28694128 | Nie et al. (2019) |
| miR-222-3p        | ING5                | neg             | 32206065 | Han et al. (2020) |
| miR-222-3p        | CDKN1B(P27)         | neg             | 28854428 | Xu et al. (2017) |
| miR-222-3p        | TIMP3               | neg             | 28854428 | Xu et al. (2017) |
| miR-223-3p        | ITGB3               | neg             | 30507047 | Liu et al. (2019a) |
| miR-223-3p        | MLC2                | neg             | 27121304 | Zeng et al. (2016) |
| miR-223-3p        | PARP1               | neg             | 26084306 | Meloche et al. (2015b) |
| miR-223-3p        | RHOB                | neg             | 27121304 | Zeng et al. (2016) |
| miR-223-3p        | BMPR2               | neg             | 29884909 | Zhang X et al. (2018) |
| miR-26b-5p        | CCND1               | neg             | 2.7322082 | Wang P et al. (2016) |
| miR-26b-5p        | CTGF                | neg             | 2.7322082 | Wang R et al. (2016) |
| miR-26b-5p        | CTGF                | neg             | 28816418 | Zhou et al. (2018) |
| miR-27a-3p        | PPARγ               | neg             | 24244514 | Kang B.-Y et al. (2013) |
| miR-27a-3p        | PPARγ               | neg             | 28484848 | Xie et al. (2017) |
| miR-27a-3p        | SMAD5               | neg             | 31004656 | Liu et al. (2019b) |
| miR-27b-3p        | PPARγ               | neg             | 25795136 | Bi et al. (2015) |
| miR-27b-3p        | PPARγ               | neg             | 28484848 | Xie et al. (2017) |
| miR-29a-3p        | KCNA5               | neg             | 31553827 | Babicheva et al. (2020) |
| miR-301a-3p       | LRP8                | neg             | 26556914 | Bertero et al. (2015) |
| miR-301a-3p       | PPARγ               | neg             | 24960162 | Bertero et al. (2014) |
| miR-301a-3p       | PPARγ               | neg             | 26556914 | Bertero et al. (2015) |
| miR-301b-3p       | LRP8                | neg             | 26556914 | Bertero et al. (2015) |
| miR-301b-3p       | PPARγ               | neg             | 24960162 | Bertero et al. (2014) |
| miR-301b-3p       | PPARγ               | neg             | 26556914 | Bertero et al. (2015) |
| miR-30a-5p        | YKL-40              | neg             | 31155141 | Tan et al. (2019) |
| miR-30c-5p        | PPARγ               | neg             | 25882492 | Xing et al. (2015) |
| miR-328-3p        | CaV1.2              | neg             | 23292900 | Guo et al. (2012) |
| miR-328-3p        | IGF1R               | neg             | 23292900 | Guo et al. (2012) |
| miR-328-3p        | IGF1R               | neg             | 31477557 | Xiong X.-Q et al. (2019) |
| miR-328-3p        | PIM-1               | neg             | 27448984 | Qian et al. (2016) |
| miR-339-5p        | FRS2                | neg             | 28947594 | Chen J et al. (2017) |
| miR-34-5p         | PDGFRα              | neg             | 27302634 | Wang P et al. (2016) |
| miR-34a-3p        | MIEF1               | neg             | 29431843 | Chen K.-H et al. (2018) |
| miR-34a-3p        | MIEF2               | neg             | 29431843 | Chen T et al. (2018) |
| miR-361-5p        | ABCA1               | neg             | 29039076 | Zhang Y et al. (2018) |
| miR-424-5p        | FGFR2               | neg             | 23263626 | Kim et al. (2013) |
| miR-424-5p        | FGFR2               | neg             | 24960162 | Bertero et al. (2014) |
| miR-424-5p        | FGFR3               | neg             | 23263626 | Kim et al. (2013) |
| miR-424-5p        | RICTOR              | neg             | 29102771 | Takagi et al. (2018) |
| miR-449a-5p       | MYC                 | neg             | 30715622 | Zhang et al. (2019a) |
| miR-454-3p        | LRP8                | neg             | 26565914 | Bertero et al. (2015) |
| miR-454-3p        | PPARγ               | neg             | 26565914 | Bertero et al. (2015) |
| miR-4632-3p       | CJUN                | neg             | 28701355 | Qian et al. (2017) |
| miR-495-3p        | VEZF1               | neg             | 31030195 | Fu et al. (2019) |
| miR-497-5p        | CDKN2B(P15)         | neg             | 31570272 | Lee and Kang, (2019) |
| miR-503-5p        | FGFR2               | neg             | 23263626 | Kim et al. (2013) |
| miR-503-5p        | FGFR2               | neg             | 24960162 | Bertero et al. (2014) |
| miR-503-5p        | FGFR1               | neg             | 23263626 | Kim et al. (2013) |
| miR-503-5p        | RICTOR              | neg             | 29102771 | Takagi et al. (2018) |
| miR-637           | CDK6                | neg             | 27204186 | Sang et al. (2016) |
| miR-665           | CDKN1A(P21)         | neg             | 31370272 | Lee and Kang, (2019) |
| miR-760           | TLR4                | neg             | 30226538 | Yang et al. (2018) |
| miR-92b-3p        | USP28               | neg             | 30149918 | Hao et al. (2018) |
| miR-942-5p        | CCND1               | neg             | 31593832 | Zhou et al. (2019) |
| miR-96-5p         | S-HT1B              | neg             | 25871906 | Wallace et al. (2015) |
| miR-98-5p         | ALK1                | neg             | 31322161 | Li et al. (2019) |
| miR-98-5p         | ET-1                | neg             | 26098770 | Kang et al. (2016) |
| MYC               | miR-19a-3p          | pos             | 17943719 | Schulte et al. (2008) |

(Continued on following page)
subnetworks of the miR-130/301 family were involved in multiple biological behaviors, such as proliferation, apoptosis, and migration in PASMCs, PAECs, and PAFs. In addition, these subnetworks also mediated the crosstalk of these pulmonary artery cells.

In PASMCs, the miR-130/301 family is involved in many regulatory axes. Among them, the POU5F1/miR-130/301 family/PPARγ axis, which regulates the expression of miR-204-5p and miR-21-5p, is the most explicitly elaborated axis. According to our studies, the identified target genes of the two miRNAs in PASMCs include BRD4, FOXM1, PSCD4, PTEN, RUNX2, and SHP2, which control cell proliferation, apoptosis, differentiation, and mitochondrial function (Courboulin et al., 2011; Meloche et al., 2015a; Green et al., 2015, 2017; Ruffenach et al., 2016; Liu et al., 2017; Bourgeois et al., 2018a). In addition to the miR-130/301 family, miR-27a/b-3p, which is regulated by NF-κB (Xie et al., 2017), can also act as an upstream controller of PPARγ in PASMCs. Interestingly, the subnetwork analysis indicates that the miR-130/301 family indirectly promotes HIF-1α expression by sustaining the RUNX2 level (Ruffenach et al., 2016). Conversely, HIF-1α induces the expression of miR-27a-3p, which depresses the level of PPARγ (Camps et al., 2014). Thus, a feedback loop with PPARγ and HIF-1α forms. This loop leads to a persistent pathological status. Moreover, as a crucial pathogenic molecule for PH, HIF-1α can function through several miRNAs, including miR-145-5p, miR-19a-3p, miR-195-5p, miR-210-3p, miR-223-3p, and miR-361-5p, to regulate the expression of downstream proteins, eventually causing abnormal cellular behaviors (Agrawal et al., 2014; Gou et al., 2012; Meloche et al., 2015b; Zeng et al., 2018; Zhang X et al., 2018, Zhang H et al., 2019; Zhao et al., 2019; Figure 8A).

In PAECs, the miR-130/301 family also plays an important role. The POU5F1/miR-130/301 family/PPARγ axis indirectly regulates the expression of ET-1 and FGF2 via miR-98-5p and

### Table 2 (Continued) List of network interactions.

| Upstream molecule | Downstream molecule | Interaction typea | PMID          | Reference                |
|-------------------|---------------------|-------------------|---------------|--------------------------|
| MYC               | miR-19b-3p          | pos               | 17943719      | Schulte et al. (2008)     |
| MYC               | miR-34a-3p          | neg               | 18066065      | Chang et al. (2005)       |
| NFATC3            | miR-23a-3p          | pos               | 19574461      | Lin et al. (2008)         |
| NF-κB             | miR-130a-3p         | pos               | 26755990      | Li Q et al. (2017)        |
| NF-κB             | miR-210-3p          | pos               | 25541039      | Liu et al. (2014)         |
| NF-κB             | miR-27a-3p          | pos               | 28484848      | Xie et al. (2017)         |
| NF-κB             | miR-27b-3p          | pos               | 28484848      | Xie et al. (2017)         |
| PPARP-1           | STAT3               | pos               | 24270264      | Mello et al. (2014)       |
| PAXIP1-AS1        | Pxn                 | pos               | 30450722      | Jandl et al. (2019)       |
| POU5F1            | miR-130a-3p         | pos               | 24960162      | Bertero et al. (2014)     |
| POU5F1            | miR-130b-3p         | pos               | 26656914      | Bertero et al. (2015)     |
| POU5F1            | miR-130b-3p         | pos               | 26656914      | Bertero et al. (2015)     |
| POU5F1            | miR-301a-3p         | pos               | 26656914      | Bertero et al. (2015)     |
| POU5F1            | miR-301b-3p         | pos               | 26656914      | Bertero et al. (2015)     |
| POU5F1            | miR-454-3p          | pos               | 26656914      | Bertero et al. (2015)     |
| PPARγ             | APLN                | pos               | 24960162      | Bertero et al. (2014)     |
| PPARγ             | miR-204-5p          | pos               | 24960162      | Bertero et al. (2014)     |
| PPARγ             | miR-21-5p           | neg               | 26208695      | Green et al. (2015)       |
| PPARγ             | miR-21-5p           | neg               | 28522588      | Green et al. (2017)       |
| PPARγ             | miR-27a-3p          | neg               | 24244514      | Kang K et al. (2013)      |
| PPARγ             | miR-98-5p           | pos               | 28696770      | Kang et al. (2016)        |
| PPARγ             | POU5F1              | pos               | 26656914      | Bertero et al. (2015)     |
| RUNX2             | HIF-1α              | pos               | 27149112      | Ruffenach et al. (2016)   |
| RXR-α             | miR-193-3p          | neg               | 24963038      | Sharma et al. (2014)      |
| SMLR              | miR-141-5p          | neg               | 32559140      | Le et al. (2020)          |
| STAT3             | miR-17-5p           | pos               | 19390056      | Brock et al. (2009)       |
| STAT3             | miR-204-5p          | pos               | 23975026      | Xu et al. (2013)          |
| STAT3             | miR-20a-5p          | pos               | 19390056      | Brock et al. (2009)       |
| STAT3             | miR-34a-3p          | neg               | 24642471      | Rokavec et al. (2014)     |
| TGF-β1            | miR-143-3p          | pos               | 2,6311719     | Deng et al. (2015)        |
| TGF-β1            | miR-199a-3p         | pos               | 20705240      | Davis et al. (2010)       |
| TGF-β1            | miR-21-5p           | pos               | 20705240      | Davis et al. (2010)       |
| TUG1              | miR-328-3p          | neg               | 31679623      | Wang et al. (2019)        |
| TYKIRIL           | PDGFRβ              | pos               | 32634060      | Zehnder et al. (2020)     |
| UCA1              | ING5                | neg               | 30553369      | Zhu et al. (2019a)        |
| ZEB1              | miR-200c-3p         | neg               | 18829540      | Bracken et al. (2008)     |

*a: pos: positive interaction, neg: negative interaction.*
FIGURE 6 | Functional analyses of the ncRNAs. (A,B) The top 10 enriched GO biological process terms and KEGG pathways in all cell types. (C,D) The top 10 enriched GO biological process terms and KEGG pathways in PASMCs. (E,F) The top 10 enriched GO biological process terms and KEGG pathways in PAECs. Node sizes indicate the number of genes enriched in functional clusters. Node colors are related to q values. GO: gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.
miR-424/503-5p, respectively (Kim et al., 2013; Zhang Y et al., 2018). The roles of ET-1 and FGF2 in PH are well established. ET-1 is synthesized primarily in endothelial cells and mediates pulmonary artery cell proliferation, migration, and constriction through two distinct G protein-coupled receptors: ETA and ETB (Clozel, 2016). Previous studies suggest that excessive FGF2 expression promotes PAEC proliferation by activating ERK1/2 and inhibits apoptosis by inducing BCL2 and BCL-xL activity (Tu et al., 2011). Furthermore, miR-130a-3p controls the level of BMPR2, which triggers idiopathic pulmonary artery hypertension (IPAH) and is involved in the development of other types of PH (Li Q et al., 2017). Considering that miRNAs from the same family have a homologous seed region sequence, other members from the miR-130/301 family may also regulate BMPR2 expression. The transcription of miR-130a-3p is controlled by NF-κB in PAECs. Thus, NF-κB and BMPR2 are linked by miR-130a-3p. In addition, miR-17a-5p, miR-20a-5p, and miR-125a-5p also mediate BMPR2 expression. Besides, two members from the miR-17-92 family, miR-17a-5p and miR-20a-5p, link STAT3 to BMPR2 (Brock et al., 2009; Huber et al., 2015; Figure 8B).

In PAFs, activation of the miR-130/301 family can induce cell proliferation and extracellular matrix remodeling by inhibiting PPARγ and LRP8. Meanwhile, matrix remodeling can activate POU5F1/miR-130/301 family/PPARγ axis in PASMCs, PAECs, and PAFs, subsequently regulating downstream molecules such as miR-204-5p, miR-424-5p, miR-503-5p, and FGF2 (Bertero et al., 2015). Upregulating miR-424-5p and miR-503-5p or inhibiting FGF2 in PAECs can repress PASMC and PAF proliferation induced by conditioned media from PAECs, indicating that these molecules are involved in the crosstalk among different pulmonary vascular cells. The remodeled extracellular matrix can also induce the expression of the proliferative miRNA, miR-27a/b-3p, in PACES, and PASMCs, as well as the expression of the vasoconstrictor ET-1, and the inflammatory cytokine IL-6 in PACES (Bertero et al., 2014; Bertero et al., 2015; Figure 9).

**4 ENVIRONMENTAL FACTORS IN PULMONARY HYPERTENSION**

Noncoding RNA interference is an important epigenetic mechanism. Recent evidence has identified the roles of epigenetic changes in the development of PH. These changes link the pathogenic genes of PH and environmental factors such as hypoxia, virus infection, and air pollution (Gamen et al., 2016). For example, BMP2 is a transmembrane serine/threonine kinase receptor, which is essential for vascular homeostasis. Although mutations in the BMP2 gene account for a considerable portion of patients with familial pulmonary artery hypertension (FPAH), only 20–30% of carriers with mutations in this gene suffer from PH, indicating that other
factors contribute to the onset of the disease (Orriols et al., 2017; Zhao et al., 2019). According to our network, hypoxia can induce the expression of several miRNAs by HIF-1α, such as, miR-145-5p, miR-19a-3p, miR-191-5p, miR-214-3p, and miR-27a-3p (Agrawal et al., 2014; Camps et al., 2014; el Azzouzi et al., 2013; Song et al., 2014; Zhao et al., 2019). Among them, miR-191-5p can increase cell proliferation, impair apoptosis, and induce phenotypic alteration through inhibiting BMPR2

FIGURE 8 | The miR-130/301 family subnetworks in (A) PASMCs, (B) PAECs, and (C) PAFs. This miRNA family was involved in multiple processes, such as cell proliferation, apoptosis, migration, endothelial contraction, and matrix remodeling.
expression, subsequently contributing to vascular remodeling (Song et al., 2014). Therefore, the HIF-1α/miR-191-5p/BMPR2 axis reveals the connection between hypoxia and BMPR2 expression and partially explains the incomplete penetrance of BMPR2 mutations in FPAH.

5 POTENTIAL APPLICATIONS OF NON-CODING RNAs

Ultimately, studies on molecular mechanisms aim to inform clinical practices. NcRNAs are potential diagnostic biomarkers for PH. For example, circRNAs are not easily degraded, making them ideal serum biomarkers. Zhang et al. reported hsa_circ_0068481 overexpression in the serum from patients with IPAH. Furthermore, hsa_circ_0068481 expression is significantly correlated with 6-min walk distance, N-terminal pro-B-type natriuretic peptide, H2S, pulmonary hypertension risk stratification, right heart failure, and survival rate (Zhang et al., 2019a). However, because of the absence of an associated molecular mechanism, this circRNA was not included in our networks. NcRNAs may also act as potential therapeutic targets for PH. For example, Rothman et al. identified downregulation of miR-140-5p in a rat PH model. In vitro, miR-140-5p mimics suppressed PASMC proliferation and migration. In vivo, miR-140-5p mimics prevented the progression of established PH in rats (Rothman et al., 2016). The results are encouraging. However, ncRNA therapy is far from being applied in clinical settings, since a ncRNA may have diverse biofunctions. This means that when used as a therapeutic agent, a ncRNA may cause adverse effects, some of which may even be life-threatening. In our opinion, carefully selected ncRNA targets and well-designed action sites can be helpful to avoid such adverse effects. These measures require a comprehensive and in-depth understanding of the mechanisms of ncRNAs in diseases. In this study, we constructed networks to demonstrate the current findings on ncRNAs from studies performed in PH patients and animal models. However, shortcomings of these studies, including the paucity of human data, sex bias, and heterogeneity of animal models, limit the translation of these findings into applications for human disease. Therefore, further studies should be performed to confirm these findings in different animal models and patient cohorts of PH. Additionally, large, well-designed, and unbiased clinical studies are required to illuminate further application of ncRNAs.

6 CONCLUSION

The roles of ncRNAs in PH remained unclear. In this study, we performed an extensive literature search and adopted uniform and strict criteria for the selection of each article to avoid biased
outcomes. The ncRNA networks were constructed by assembling ncRNAs and their interacting RNAs or genes from included articles. These networks provide a better understanding of the roles of ncRNAs in PH and can be helpful in elucidating the potential clinical applications of ncRNAs.

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