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RESEARCH ARTICLE

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MicroRNA expression alteration in chronic thromboembolic pulmonary hypertension: A systematic review

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Abstract

Chronic thromboembolic pulmonary hypertension (CTEPH) is marked by persistent blood clots in pulmonary arteries, leading to significant morbidity and mortality. Emerging evidence highlights the role of microRNAs (miRNAs) in pulmonary hypertension, though findings on miRNA expression in CTEPH remain limited and inconsistent. This systematic review evaluates miRNA expression changes in CTEPH and their direction. Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, we registered our protocol in International Prospective Register of Systematic Reviews (CRD42024524469). We included studies on miRNA expression in CTEPH with comparative or analytical designs, excluding nonhuman studies, interventions, non-English texts, conference abstracts, and editorials. Databases searched included PubMed, EMBASE, Scopus, CENTRAL, and ProQuest. The Quality Assessment of Diagnostic Accuracy Studies-2 tool assessed bias risk, and results were synthesized narratively. Of 313 unique studies, 39 full texts were reviewed, and 9 met inclusion criteria, totaling 235 participants. Blood samples were analysed using quantitative real time polymerase chain reaction. Seven miRNAs (miR-665, miR-3202, miR-382, miR-127, miR-664, miR-376c, miR-30) were uniformly upregulated, while nine (miR-20a-5p13, miR-17-5p, miR-93-5p, miR-22, let-7b, miR-106b-5p, miR-3148, miR-320-a, miR-320b) were downregulated in CTEPH patients. Two upregulated miRNAs (miR-127 and miR-30a) were consistently associated with previous evidence in the mechanism inducing the development of CTEPH, and five downregulated miRNAs (miR-20-a, miR-17-5p, miR-93-5p, let-7b, miR-106b-5p) were associated with a protective effect against CTEPH. We also identified gaps in the literature where the evidence for five upregulated miRNAs (miR-665, miR-

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3202, miR-382, miR-664 and miR-376c) and four downregulated miRNAs (miR-22, miR-3148, miR-320-a, and miR-320b) in CTEPH is conflicting. Our findings offer insights into the role of miRNAs in CTEPH and underscore the need for further research to validate these miRNAs as biomarkers or therapeutic targets.

KEYWORDS

chronic thromboembolic pulmonary hypertension, differentially expressed miRNAs, miRNA, pulmonary artery hypertension

INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH), also known as type IV pulmonary hypertension, is a form of pulmonary hypertension caused by persistent blood clots obstructing the pulmonary arteries. It is characterized by thromboembolic material, impaired fibrinolysis, altered vascular remodeling, and endothelial dysfunction.[1] A systematic review conducted by Gall et al. on CTEPH epidemiology provides insight into its incidence, that was reported to be 3–5 per 100,000 in Europe and the United States, and 1.94 per 100,000 in Japan. However, it is evident that the disease is significantly underdiagnosed. It is estimated that only 16% of cases were diagnosed in 2015, and that by 2025, the diagnosis rate would not improve significantly, with only 28% of CTEPH cases being diagnosed.[2]

Despite the availability of treatments such as pulmonary endarterectomy (PEA), balloon pulmonary angioplasty (BPA), and targeted pharmacotherapies, these interventions are not universally effective and some treatment, such as BPA, while is effective, is associated with serious complications.[3] As a result, CTEPH has a high mortality rate and patients with the condition often experience progressive disease and reduced quality of life.[4]

MicroRNAs (miRNAs) are small, noncoding RNA molecules that play crucial roles in regulating gene expression at the posttranscriptional level. They influence various cellular processes, including proliferation, apoptosis, and tissue remodeling.[5] Due to their role, previous evidence indicated that miRNAs have the potential to become diagnostic and prognostic biomarker, or even therapeutic agent.[6,7] In the context of pulmonary hypertension, miRNAs have been implicated in its pathophysiology, such as inflammation and vascular remodeling.[8] It was postulated that dysregulation of specific miRNAs could contribute to the development and progression of CTEPH, suggesting that these molecules may serve as potential biomarkers for diagnosis and targets for therapeutic intervention.[9]

However, despite advancement of knowledge, there remains a significant gap in the understanding of miRNA expression in CTEPH. Existing studies have reported inconsistent findings regarding which miRNAs are dysregulated in the disease, their direction of change, and their specific roles in disease pathogenesis.[9,10] This inconsistency highlights the need for a comprehensive synthesis of current research data to identify the alteration of miRNA expression in CTEPH. We performed a systematic review to evaluate the change in miRNA expression in patients with CTEPH compared to subjects without CTEPH and to assess the direction of change of altered miRNA expression.

METHODS

Our study adheres to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. The protocol has been registered in the International Prospective Register of Systematic Reviews as CRD42024524469.

Eligibility criteria, search strategy, and study selection

We conducted an online search of databases, which includes PubMed®, EMBASE, Scopus, CENTRAL, and ProQuest. The search was performed using the following query, (“CTEPH” OR “chronic pulmonary embolism*“ OR “chronic pulmonary thromboembolism*“ OR “chronic thrombo-embolic pulmonary hypertension*“ OR “CTEPH*“OR “group 4 pulmonary hypertension*“ OR “group IV Pulmonary Hypertension*“ OR “group 4 ph*“ OR “group IV ph*“) AND (“microRNAs”[MeSH Terms] OR “miRNAs” OR “MicroRNAs”[Mesh] OR “MicroRNA*“ OR “Micro RNA*“ OR “pri-miRNA*“ OR “pre-miRNA*“ OR “noncoding RNA*“ OR “noncoding RNA*“ OR “ncRNA*“). We tailored the search strategy for each database.

Studies were included if they focused on miRNA expression in subjects with CTEPH compared to controls and were conducted with comparative or analytical study designs. We excluded the following studies: (1) not conducted in humans; (2) not including healthy subjects (people without CTEPH) as comparator; (3) focusing on intervention; (4) not written in English; (5) conference abstracts and editorials. We did not restrict trials by year of publication.

Data extraction and outcomes of interest

The search results were entered into EndNote 20 (Clarivate Analytics) to remove duplicates and to provide information on the number of studies per database. Two authors (HS and LKS) independently screened the records according to the inclusion and exclusion criteria. Any disagreements were resolved by discussion with the third reviewer (HH). We used Microsoft® Excel for Mac Version 16.74 to extract information from eligible records in the form of spreadsheet.

Eligible records were reviewed and the following information was extracted: (1) first author; (2) year of publication; (3) study title; (4) mean or median age of subjects; (5) number of subjects; (6) source of sample collection; (7) confirmation assay used to quantify miRNA; (8) changes in miRNA expression; (9) miRNA expression measure.

We defined the primary outcome as changes in miRNA expression in subjects with CTEPH compared to controls. The effect of the measure is reported according to the methods used in each of the studies, that is, as relative expression to control.

Bias assessment

We used the Quality Assessment of Diagnostic Accuracy Studies-2 tool to assess publication bias. The tool comprises seven domains, three of which pertain to bias (patient selection, index test, reference standard, flow and timing) and three to applicability (patient selection, index test, and reference standard. Each domain consists of several questions to assess bias and applicability.[11]

A summary of the overall bias of each study was categorized into low, unclear, and high risk. A study was defined as having a low risk of bias if the answer to each question was "yes". Conversely, a study was defined as having a high risk of bias if the answer to each question was "no". If the answer to a question could not be determined due to limitations in the information provided, the study was defined as having an unclear risk of bias. Two authors (HS and LKS) assessed each study and disagreements were resolved by a third author (HH).

Data synthesis

The data synthesis was conducted in accordance with the qualitative synthesis approach, as meta-analysis was not feasible due to the lack of sufficient data on miRNA expression changes or diagnostic accuracy. The extracted data from the included studies were compiled into a summary table, which detailed the study characteristics, sample sources, miRNA quantification methods, and changes in miRNA expression. The primary outcome was defined as the changes in miRNA expression levels in subjects with CTEPH compared to controls.

RESULTS

Study selection and characteristics

Our literature search yielded 313 unique studies. Following the screening of abstracts, 39 full texts were reviewed and 9 studies were subsequently included in the systematic review (Figure 1). All studies collected samples from blood[12–19]; with four utilizing plasma,[12,16,18,19] one using serum,[15] and four not specifying the type of sample used.[13,14,17,20] Table 1 provides a summary of the characteristics of the included studies.

The method of miRNA isolation, concentration and integrity assessment were varied between studies. For the isolation of miRNA, the reagents used were miRNeasy Serum/Plasma kit (QIAGEN, Hilden, Germany)[12]; RNAprep Pure Blood kit (Qiangen Biotech Co., Ltd., China),[13–15,17] TRI Reagent® (Sigma Aldrich, St. Louis, United States),[16,18] and TRIzol reagent (Thermo Fisher Scientific, United States).[20] Furthermore, the utilization of a purification kit, namely mirVanaTM miRNA (AM1561, Ambion, United States) has been documented in a number of studies.[13,14,17]

The concentration of miRNAs was assessed using two methods: the Qubit® Fluorometer (Invitrogen, Life Technologies)[12,14,17] and the Nanodrop ND1000 spectrophotometer (Thermo Fisher Scientific).[13,20] The aforementioned five studies conducted integrity analysis with the Agilent 2100 Bioanalyzer (Agilent Technologies).[12–14,17,20] It should be noted, however, that four studies did not specify the methods used to assess miRNA concentration and integrity.[15,16,18,19]

Eight studies employed quantitative real-time polymerase chain reaction (qRT-PCR) as a confirmatory assay to quantify miRNA expression,[12–19] whilst one

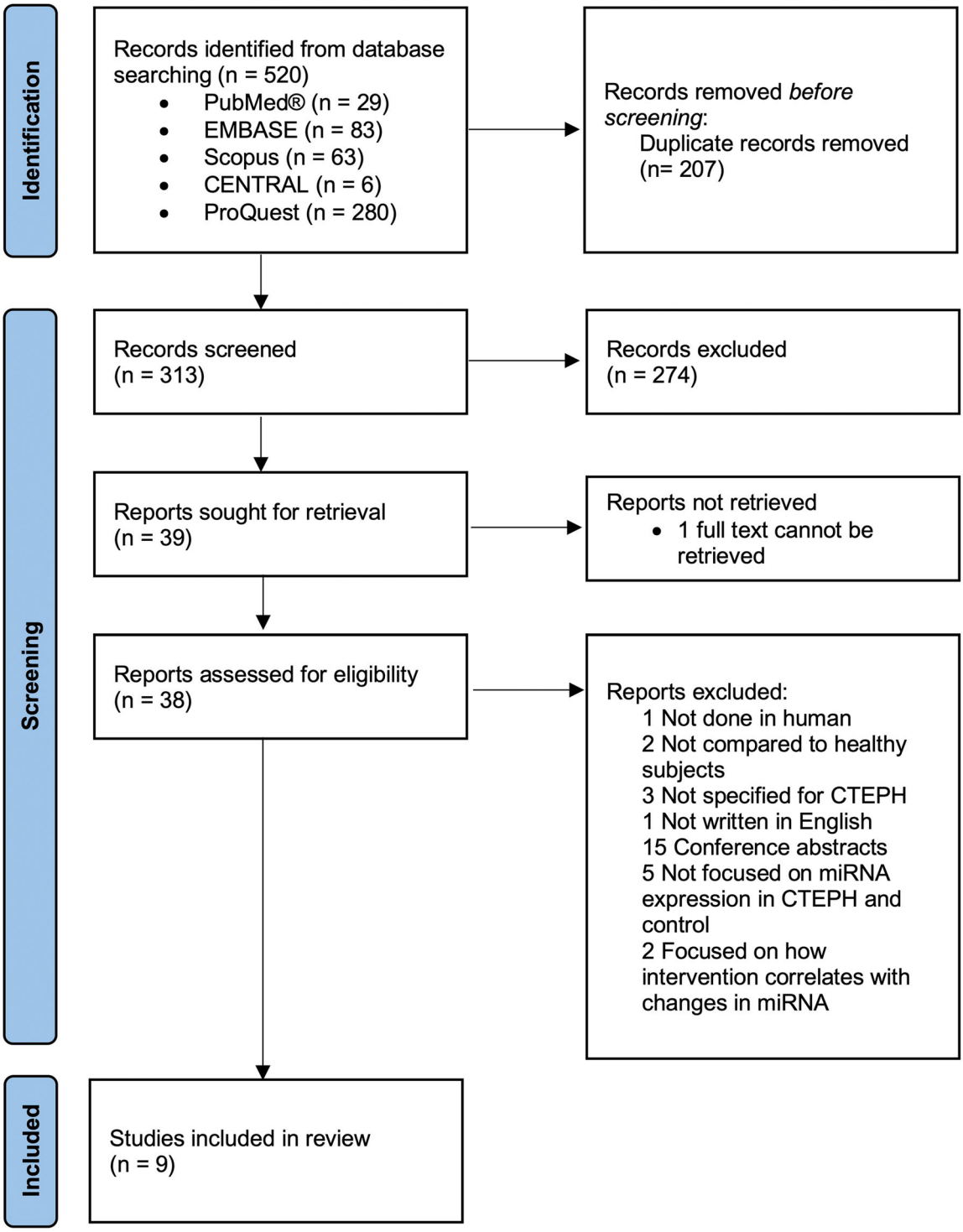

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flowchart.

study performed only RT-PCR followed by mathematical calculation $2^{-\Delta\Delta Ct}$ to quantify relative miRNA expression.[20] Seven studies measured the changes in miRNA expression as a relative expression to the control group.[13,14,16–20] However, one study did not specify the outcome measure,[12] while another employed a logarithmic transformation of miRNA levels.[15]

Risk of bias assessment

The summary of the quality assessment is presented in Figure 2. Six studies were classified as high risk in the domain of patient selection due to their case-control design. Nevertheless, all other domains were assessed to be of low risk of bias.

TABLE 1 Study characteristics.

Study	Age (Mean ± SD)	*N* Control	*N* CTEPH	Country	Sample source; Anticoagulant	Confirmative assay	miRNAs studied and results
Fabro et al. (2021)[12]	Median (IQR) Control: 42 (32–52) CTEPH: 53 (42–68)	13	14	Brazil	Blood (source not specified), centrifuged at 1100 g at 4℃ for 10 min, followed by 1300 g at 4℃ for 20 min to collect plasma; EDTA	qRT PCR	**Downregulated:** hsa miR-320-a. miRNA-320-a expression ≤2.58 was associated with CTEPH **No significant difference:** miR-let-7i-5p, miR-320b-1, miR-320b-2, miR-1291
Miao et al. (2022)[13]	Control: NA CTEPH: 61.0 ± 6.8	8	8	China	Peripheral venous blood; Anticoagulant not specified	qRT-PCR	**Downregulated:** miR-20a-5p; miR-17-5p; miR-93-5p **Upregulated**: miR-665; miR-3202
Miao et al. (2017)[14]	Control: 58.2 ± 9.17 CTEPH: 58 ± 13.77	5	4	China	Blood (source not specified); Anticoagulant not specified	qRT-PCR	**Downregulated**: miR-3148
Iwatani et al. (2021)[15]	Control: 67.4 ± 12.0 CTEPH: 65.2 ± 8.9	30	22	Japan	Peripheral venous blood, centrifuged at 1000 g for 15 min to collect serum; Anticoagulant NA	qRT-PCR	**No significant difference:** miR140-3p and miR485-5p
Guo et al. (2014)[16]	Control: 49.6 (9.5) CTEPH: 51.9 (11.6)	40	40	China	Blood (source not specified), centrifugated at 1000 g for 10 min to collect plasma; EDTA	qRT-PCR	**No significant difference:** miR-602 **Downregulated:** miR-22, let-7b
Gong et al. (2021)[17]	Control: 56.13 ± 4.49 CTEPH: 61.00 ± 6.82	8	8	China	Peripheral blood (source not specified); Anticoagulant not specified	qRT-PCR	**Downregulated**: miR-17-5p; miR-106b-5p; miR-93-5p **Upregulated**: miR-3202; miR-665
Miao et al. (2020)[18]	NA	10	10	United States of America	Blood (source not specified), centrifugated at 1000 g for 10 min to collect plasma; EDTA	qRT-PCR	**Downregulated**: miR- 106b-5p; miR-17-5p **Upregulated**: miR-665; miR-3202
Lipps et al. (2019)[19]	Control: 56 (NA) CTEPH: 60 (NA)	20	20	Germany	Blood (source not specified), centrifugated at 3000 g for 10 min to collect plasma; EDTA	qRT-PCR	**No significant difference**: miR-150 **Upregulated**: miR-382; miR-127; miR-664; mIR-376c; miR-30a
Xu et al. (2022)[20]	Control: 48.8 ± 9.54 CTEPH: 53.42 ± 11.90	30	19	Not specified	Peripheral venous blood; Anticoagulant not specified	RT-PCR followed by mathematical calculation $2^{-\Delta\Delta Ct}$ to calculate relative expression	**Downregulated**: miR-320b

Abbreviations: bqRT-PCR, quantitative real time polymerase chain reaction; cRT-PCR, real time polymerase chain reaction; Ct, cycle threshold; EDTA, ethylenediaminetetraacetic acid.

FIGURE 2 Risk of bias assessment.

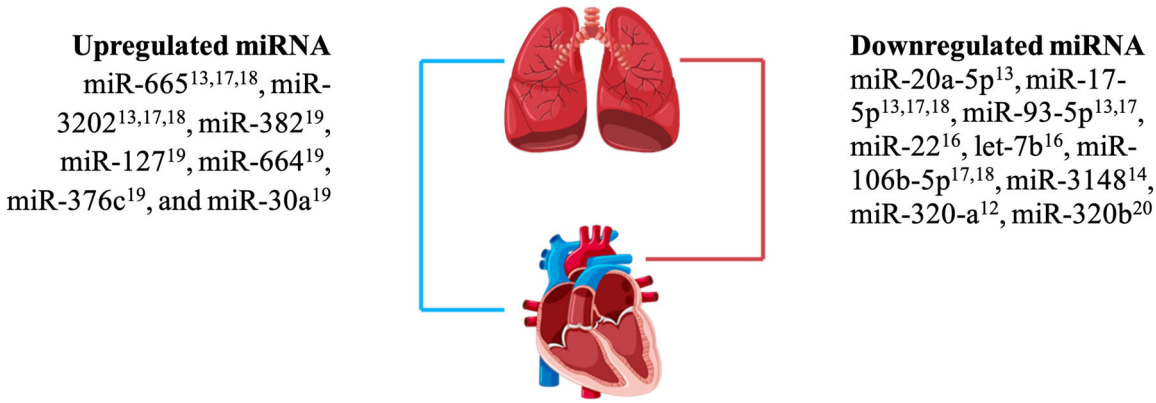

FIGURE 3 miRNA changes in chronic thromboembolic pulmonary hypertension (CTEPH) patients compared to healthy subjects.

Changes in miRNA expression

Seven miRNAs were reported to be upregulated in patients with CTEPH, namely miR-665,[13,17,18] miR-3202,[13,17,18] miR-382,[19] miR-127,[19] miR-664,[19] miR-376c,[19] and miR-30a.[19] In contrast, nine following were found to be downregulated: miR-20a-5p,[13] miR-17-5p,[13,17,18] miR-93-5p,[13,17] miR-22,[16] let-7b,[16] miR-106b-5p,[17,18] miR-3148,[14] miR-320-a,[12] miR-320b[20] (Figure 3).

For the upregulated miRNA, two miRNAs that is miR-665 and miR-3202 were reported to be upregulated in three studies, and the other miRNAs were reported in one study each. Similarly, the downregulation of miR-17-5p were reported in three studies, miR-106b-5p and miR-93-5p in two studies and the other were reported in a single study. Figure 4 illustrates the frequency with which each miRNA was reported in the study.

DISCUSSION

The dysregulation of multiple miRNAs in patients with CTEPH highlights the possibility of molecular mechanisms underlying the disease pathogenesis. Our systematic review identified no conflicting or inconsistent reports of miRNA expression. However, the exact mechanisms by which the dysregulated miRNAs affect CTEPH pathogenesis are still far from being elucidated. Plausible theories include miRNAs are associated with various cellular processes relevant to CTEPH, such as

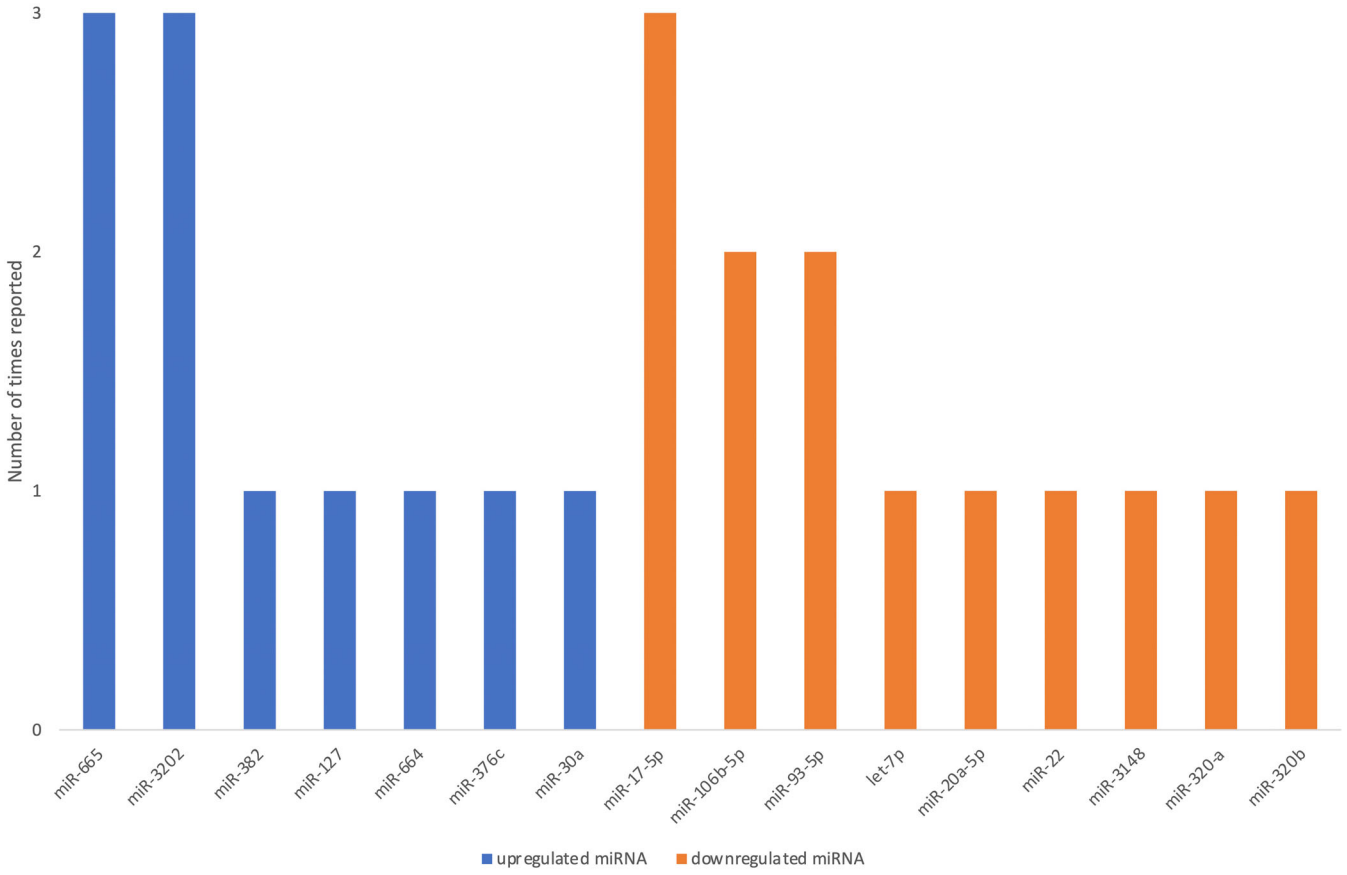

FIGURE 4 Dysregulation of miRNA by number of study reports.

vascular smooth muscle proliferation, apoptosis, inflammation, platelet activation, thrombosis, and clotting factors impairment (Table 2).

We found seven miRNAs to be upregulated, including miR-665,[13,17,18] miR-3202,[13,17,18] miR-382,[19] miR-127,[19] miR-664,[19] miR-376c,[19] and miR-30a.[19] In accordance with the prevailing theory, these miRNAs are postulated to exert a maladaptive effect, whereby upregulation is posited to induce disease development. However, as demonstrated in Table 2, the available evidence on the role of miRNAs in CTEPH is inconclusive.

Of the seven upregulated miRNAs (Table 2), only two were found to be consistent with previous research on the role of miRNAs and their association with CTEPH. These were miR-127, and miR-30a. It is postulated from the previous evidence, that these miRNAs induce CTEPH development by promoting inflammation that in turns lead to Pulmonary Artery Smooth Muscle remodeling (miR-127), dysregulates Factor VIII and Factor XI, and was also associated with acute myocardial infarction (miR-30a).[30,31] Their mechanism is also illustrated in Figure 5. The remaining five were found to be inconsistent with previous evidence (i.e., miR-665, miR-3202, miR-382, miR-664, and miR-376c), despite two of them being found to be uniformly upregulated in three records, i.e., miR-665 and miR-3202 (Figure 4).

The upregulation of those miRNAs with conflicting evidence, such as miR-665, presents a paradox as it contradicts its known protective role in CTEPH. It was postulated that uncontrolled vascular smooth muscle cell proliferation and migration increases the risk of CTEPH[48]; and the disease is associated with altered vascular remodeling and endothelial dysfunction.[1] Therefore, high levels of miR-665, which inhibits vascular remodeling (Table 2), should theoretically be protective against CTEPH.[21] However, we found that three studies reported miR-665 to be upregulated in CTEPH. Another plausible explanation for this phenomenon is that perhaps the high levels of miR-665 observed in patients with CTEPH are an adaptive response against disease progression, as shown by previous evidence that miRNAs have a multifactorial nature, including in vascular remodeling and cardiovascular disease.[49]

Conversely, several miRNAs were uniformly found to be downregulated in CTEPH, including miR-20a-5p,[13] miR-17-5p,[13,17,18] miR-93-5p,[13,17] miR-22,[16] let-7b,[16] miR-106b-5p,[17,18] miR-3148,[14] miR-320-a,[12] miR-320b.[20] Of these, miR-17-5p was reported in three studies and miR-

TABLE 2 Associated role of miRNAs and CTEPH.

miRNA	Associated role	Association with CTEPH
Upregulated miRNAs		
miR-665	Inhibits proliferation and migration of VSMCs via the targeting of FGF9, MEF2D, and the Wnt/β-catenin signaling pathway.[21]	Conflicting evidence
miR-3202	Inhibits FAIM2, leading to an increase in apoptosis[22];	Conflicting evidence
miR-382	Promotes apoptosis in various cancer cells.[23,24]	Conflicting evidence
miR-127	Promotes inflammation by activating JNK pathway, that in turns lead to PASMC remodeling.[9,25]	Inducing
miR-664	Inhibits apoptosis and promotes proliferation in osteosarcoma[26]; however, it is reported to inhibit cells proliferation in cutaneous malignant melanoma.[27]	Conflicting evidence
miR-376c	Inhibits PTCP and were inversely correlated with PAR4 activity.[28] When activated, PAR4 has procoagulant activity and enhance clot stability.[29]	Conflicting evidence
miR-30a	Associated with Factor VIII and Factor XI dysregulation,[30] upregulation is associated with acute myocardial infarction.[31]	Inducing
Downregulated miRNAs		
miR-20-a	Inhibits TF, while TF is known to initiate coagulation cascade and provoke thrombotic events.[32,33]	Protective
miR-17-5p	Promote apoptosis through Tsg101[36]	Protective
miR-93-5p	Supress VEGF gene expression,[35] where VEGF is crucial in promoting vascular proliferation and remodeling.[36]	Protective
miR-22	Inhibits vascular apoptosis and promotes vascular remodeling.[37,38]	Conflicting evidence
let-7b	Increase apoptosis in hepatocellular carcinoma[39]; inhibits vascular smooth muscle proliferation and remodeling through downregulation of IGF1.[40]	Protective
miR-106b-5p	Supresses NOR-1 expression, where NOR-1 is involved in PASMC proliferation, migration, and remodeling.[41,42]	Protective
miR-3148	Inhibits TGFβ° pathway, lead to reduced apoptosis and increased cell proliferation[43]	Conflicting evidence
miR-320-a	Significantly elevated in thrombotic diseases such as deep vein thrombosis[44]; increased platelet reactivity is associated with the interaction of WIPF1 gene in miR-320-a overexpression and associated with PASCM proliferation[45]	Conflicting evidence
miR-320b	Associated with deep vein thrombosis and acute myocardial infarction, and ICAM-1 expression.[46,47]	Conflicting evidence

Abbreviations: bFGF9, fibroblast growth factor 9; cMEF2D, myocyte enhancer factor 2D; dWnt/β, Wingless/Integrated/β; eFAIM2, Fas apoptotic inhibitory molecule 2; fJNK, Janus Kinase; gPASMC, pulmonary artery smooth muscle cells; hPTCP, phosphatidylcholine transfer protein; iPAR4, protease-activated receptor 4; jTF, tissue factor; kTsg101, tumor susceptibility gene 101; lVEGF, vascular endothelial growth factor; mIGF1, Insulin-like Growth Factor-1; nNOR-1, neuron-derived orphan receptor-1; oTGFβ, tumor growth factor β; pWIPF1, Wiskott-Aldrich Syndrome Interacting Protein Family Member 1; qICAM-1, intercellular adhesion molecule-1; SMC, vascular smooth muscle cell.

106b-5p and miR-93-5p in two studies (Figure 4). According to previous theory, these miRNAs are thought to have a protective effect, with downregulation being associated with CTEPH development.

Of the nine downregulated miRNAs, five were found to be consistent with previous research on the role of miRNAs and their association with CTEPH. These were miR-20-a, miR-17-5p, miR-93-5p, let-7b, miR-106b-5p that were postulated to be protective against CTEPH. The mechanism on which these miRNAs is associated with CTEPH is presented in Table 2. Those mechanisms include inhibits Tissue Factor (TF) and prevent thrombotic events (miR-20-a); promote apoptosis through tumor susceptibility gene 101 (miR-17-5p); suppress vascular remodeling via inhibition of Vascular Endothelial Growth Factor (miR-93-5p); increase apoptosis, and inhibit vascular smooth muscle proliferation and remodeling (let-7b); inhibit Pulmonary Artery Smooth Muscle cell proliferation, migration, and remodeling by supressing Neuron-Derived Orphan Receptor-1 (miR-105b-5p).[32–36,40–42] These mechanisms are also outlined in the Figure 5.

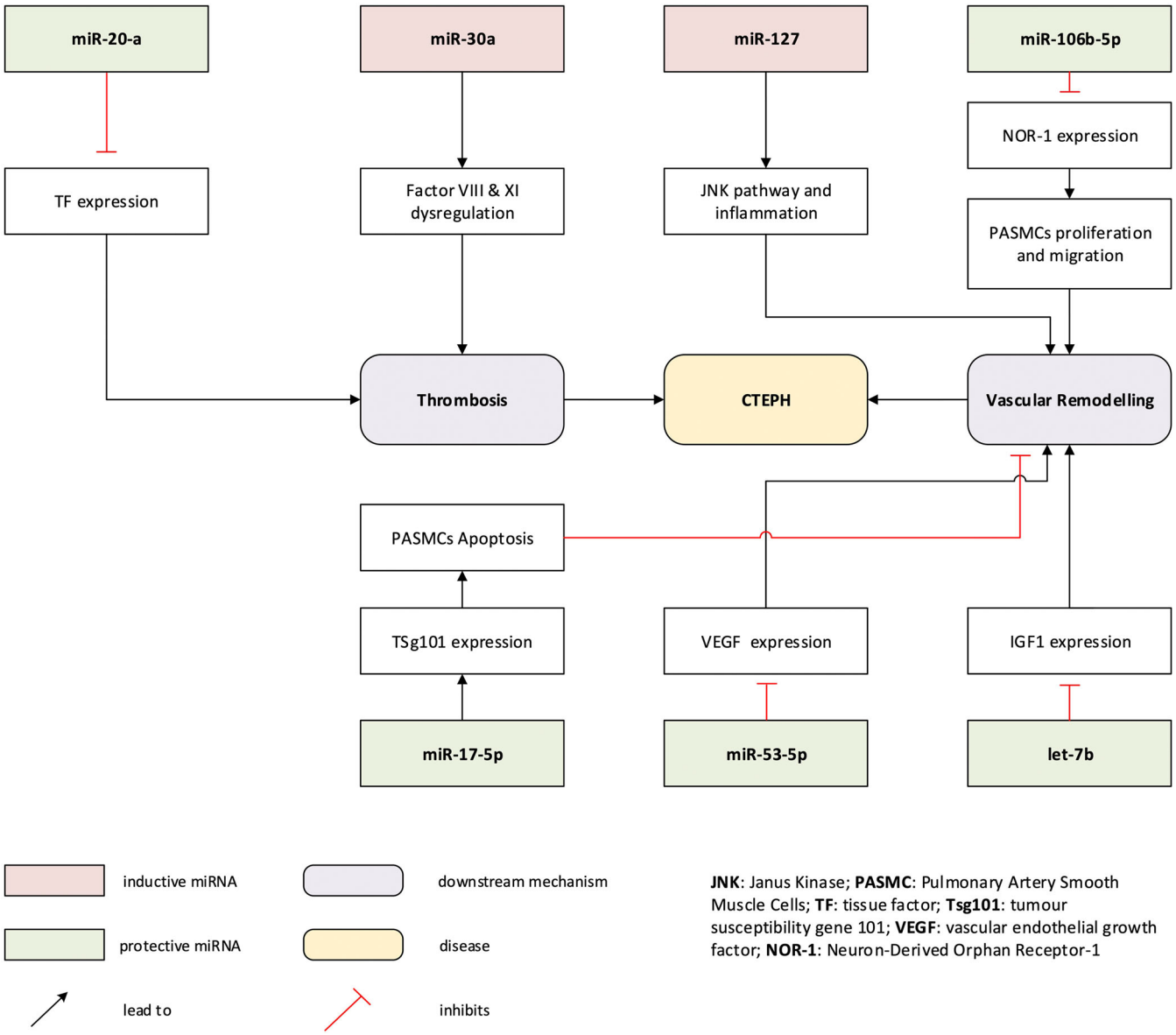

FIGURE 5 MicroRNA-mediated mechanisms in chronic thromboembolic pulmonary hypertension (CTEPH) disease modulation.

Four additional miRNAs, namely miR-22, miR-3148, miR-320-a, and miR-320b, were identified as being inconsistent with the existing evidence. In contrast with the previous hypothesis that their expression were associated with risk of developing CTEPH, these miRNAs were found to be downregulated in patient with CTEPH.[12,14,16,20]

In summary, two upregulated miRNAs (miR-127 and miR-30a) were identified as being associated with the induction of CTEPH development. Conversely, five downregulated miRNAs (miR-20-a, miR-17-5p, miR-93-5p, let-7b and miR-106b-5p) were found to have a protective effect against CTEPH. However, inconsistencies were observed in the existing literature regarding the evidence for five upregulated miRNAs (miR-665, miR-3202, miR-382, miR-664 and miR-376c) and four downregulated miRNAs (miR-22, miR-3148, mir-320-a and miR-320b) with the development of CTEPH. From these findings, it could be postulated that miRNAs may play an essential role in the development of CTEPH (Figure 5). The downstream mechanisms that were identified to be influenced with miRNAs dysregulation are thrombosis and vascular remodeling.

To date, research focusing on miRNAs that are involved in CTEPH is still scarce. To the best of our knowledge, this systematic review is the first to assess miRNAs expression change in CTEPH. Whilst our study provides new insight into the field, it has several limitations. The limitations of the evidence included in this review are primarily due to the heterogeneity of study

designs and methodologies employed across the selected studies. Furthermore, whilst case-control studies are valuable for assessing associations, they are susceptible to biases such as selection bias and confounding variables.[50] This study design is also unsuited to establish causal relationship because subjects have already been diagnosed with the disease during the study period. Therefore, it is not possible to determine the rate of disease development between exposure-positive and exposure-negative individuals in this case.[51]

Despite the considerable progress that has been made in miRNA detection, conflicting evidence regarding miRNA expression were not uncommon. This phenomenon may be influenced by a number of factors, including pre-analytical and analytical factors.[52] Furthermore, the level of miRNA expression may differ amongst different ethnic groups. This may contribute to the inconsistency in findings regarding the type of miRNA expressed or the direction of expression.[53,54]

In addition, whilst efforts were made to conduct a comprehensive search of multiple databases and adhere to PRISMA 2020 guidelines, it is possible that relevant studies may have been inadvertently excluded. The inclusion of only English-language studies may introduce language bias, potentially overlooking relevant evidence published in other languages.

Implications and future directions

Regardless of its limitations, our findings offer valuable insights into the dysregulation of miRNAs in CTEPH and identify potential targets for further investigation and therapeutic intervention. The identified miRNAs may serve as biomarkers for disease diagnosis, prognosis, or treatment response. Furthermore, elucidating their roles in disease pathogenesis could inform the development of novel therapeutic strategies.

Future research should focus on elucidating the functional implications of miRNA dysregulation in CTEPH and exploring their therapeutic potential in mitigating disease progression. Furthermore, it is recommended that efforts be made to validate the findings of our systematic review through prospective larger-scale studies and functional assays to further elucidate the roles of dysregulated miRNAs in CTEPH pathogenesis.

AUTHOR CONTRIBUTIONS

Heru Sulastomo devised the project, the main conceptual ideas, proof outline, and reviewed articles. Lucia Kris Dinarti reviewed articles and worked out the technical details. Hariadi Hariawan resolved disagreement in review and assisted data synthesis. Sofia Mubarika Haryana conducted the data synthesis. All authors contributed in manuscript writing and proofreading.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The authors have nothing to report.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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