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Recent progress in the roles of microRNAs in pulmonary arterial hypertension associated with congenital heart disease

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Abstract

Research on noncoding RNA, particularly microRNAs (miRNAs), is growing rapidly. Advances in genomic technologies have revealed the complex roles of miRNAs in pulmonary arterial hypertension (PAH) associated with congenital heart disease (CHD). It has been demonstrated that the progression of PAH associated with CHD is characterized by particular dysregulation of miRNAs and is related to cardiovascular remodeling, cell death, and right ventricle dysfunction. This review provides a comprehensive overview of the current state of knowledge regarding the involvement of miRNAs in the pathogenesis and progression of PAH associated with CHD. We commence by explaining the process of miRNA synthesis and its mode of action, as well as the role of miRNA in PAH associated with CHD. Moreover, the article delves into current breakthroughs in research, potential clinical implications, and prospects for future investigations. The review provides the insight into novel approaches for diagnosis, prognosis, and therapy of PAH associated with CHD.

Keywords: Cardiovascular abnormality, pulmonary arterial hypertension, PAH, microRNA, non-coding RNA

Introduction

Pulmonary arterial hypertension (PAH), a fatal and the most frequent complication in individuals with congenital heart disease (CHD), is characterized by a mean pulmonary arterial pressure >20 mmHg from right heart catheterization [1]. PAH associated with CHD is one of the most prevalent types of PAH, along with idiopathic PAH and PAH caused by connective tissue diseases [2,3]. A center registry in Yogyakarta, Indonesia, reported that 66.9% of adult patients with CHD from July 2012 until July 2019 developed PAH with atrial septal defect (ASD) was the most common type of CHD (73.4%) [4]. Further analysis using same registry showed that patients with PAH associated with ASD had a mortality rate of 61.9% during the follow-up period [5].

Interest in the role of microRNAs (miRNAs) throughout the emergence and progression of PAH, which also includes PAH associated with CHD, has developed over the past few years. miRNAs, which are small noncoding RNA molecules, play an important role in the regulation of gene expression by interacting with complementary sequences in the 3′ untranslated regions of their target messenger RNAs (mRNAs) [6-8]. miRNAs also play a crucial role in various fundamental biological processes, including differentiation, proliferation, apoptosis, and organogenesis; all of these factors lead to the progression of pulmonary arteriopathy [9,10].

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Moreover, miRNAs have been recognized as future biomarkers in various diseases [11-16] including PAH [17]. Patients with PAH associated with CHD have been shown to exhibit the altered expression of several miRNAs, and this variation is correlated with disease severity and prognosis [18,19]. This review aims to provide an overview of the recent progress in the research on the roles of miRNAs in PAH associated with CHD. This article provides an overview miRNA and its mechanisms of action, as well as its role in PAH associated with CHD. Additionally, it discusses recent research discoveries, potential clinical applications, and prospects for future investigations in this field.

MiRNA: Identity and function

miRNAs are a group of small RNA molecules that lack the ability to encode proteins [8]. They are pivotal in the modulating and controlling gene expression. Primary miRNAs are transcribed from DNA sequences and undergo further processing into miRNA precursors (pre-miRNAs) and mature miRNAs [20]. These molecules are approximately 19-25 nucleotides long and possess the ability to form bonds with mRNA molecules [21-23]. This interaction subsequently results in the breakdown of mRNAs or the suppression of mRNA translation (**Figure 1**). The main function of these small RNA molecules is to operate at a posttranscriptional stage through targeting and binding to certain mRNA sequences [7]. miRNAs have been demonstrated to influence the pathogenesis and advancement of a variety of diseases, including PAH, by controlling the expression of numerous genes [24-27]. The dysregulated expression of miRNAs has been found to be connected with several disease processes [28-30].

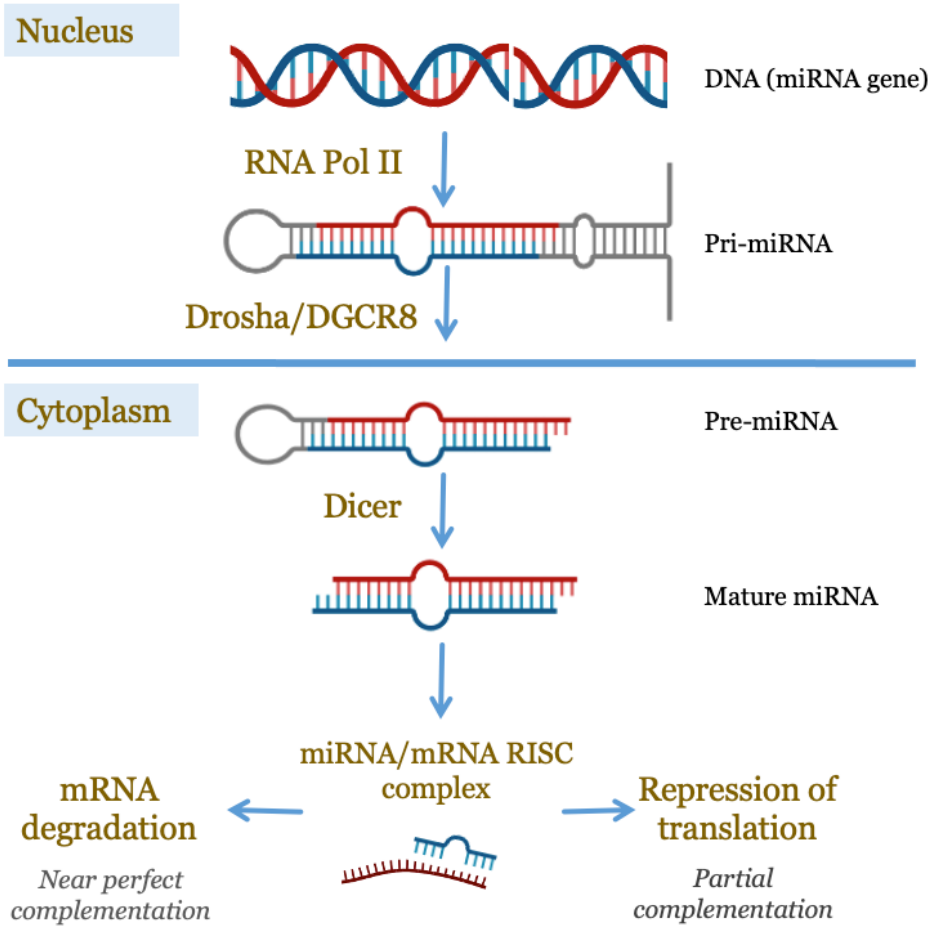

Figure 1. MicroRNA (miRNA) synthesis and mechanism of action. MiRNAs are transcribed by RNA polymerase II and undergo various maturation steps to become mature microRNA. Mature miRNA acts as a regulator by binding to target messenger RNA, resulting in entire or partial inhibition of the target genes.

Understanding the role of miRNAs in PAH

PAH associated with CHD frequently arises because of a result of left-to-right shunt abnormalities or left heart obstructive diseases that lead to precapillary or postcapillary hypertension, respectively [31,32]. Environmental variables and genetic or epigenetic influences have been postulated to contribute to its development [33]. This phenomenon is exemplified by a study that demonstrated that a subset (6%) of individuals diagnosed with PAH associated with CHD had mutations in the *BMPR2* gene [34]. Novel *NKX2-5* gene variants have been reported to influence the severity of PAH associated with ASD [35].

miRNAs are responsible for regulating the expression of genes involved in vascular remodeling, smooth muscle cell proliferation, and inflammation, all of which contribute to the development of pulmonary arteriopathy [36-38]. For instance, miR-125a, as determined by computational analysis, specifically targets the *BMPR2* gene [38]. This is confirmed by the findings of in vivo experiments, which revealed increased expression of miR-125a under hypoxic conditions, concomitant with reduced expression of the *BMPR2* gene and the tumor suppressor gene *CDKN1A* [38]. The *NKX2-5* gene executes its upstream and downstream actions via miR-19a and miR-19b (miR-19a/b) to control cardiomyocyte apoptosis and proliferation, respectively [39].

Furthermore, the biphasic modulation of cardiac remodeling and cardiomyocyte apoptosis was induced by the overexpression of miR-21 during the early phase (right ventricle hypertrophy) and downregulation of miR-21 during the late phase (right ventricle dysfunction) of PAH associated with CHD [40]. miR-21 has been shown to play an important role in the development of chronic hypoxia-induced pulmonary vascular remodeling [41]. In patients with PAH, the miR-424(322) has diagnostic and prognostic values and is correlated with disease severity markers. In addition, miR-424(322) can target proteins that directly affect heart function [42]. Although the causes of pulmonary vascular remodeling in PAH are unclear, a cancer-like notion has been developed due to various parallels to carcinogenesis [43]. Considering the substantial influence of miRNAs on gene expression and cellular functions, the dysregulation of their expression has been linked to the development of PAH associated with CHD (**Figure 2**).

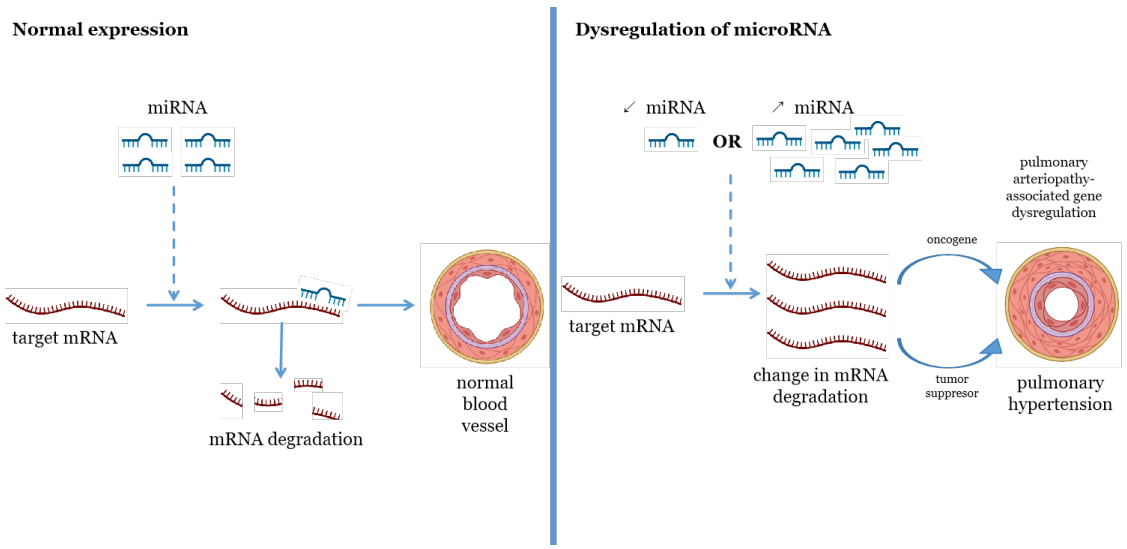

Figure 2. Regulation of genes by microRNAs (miRNAs) in pulmonary arterial hypertension (PAH) associated with congenital heart disease (CHD). This figure illustrates that in PAH associated with CHD, there are alterations in the expression of miRNAs. These changes result in both an increase and decrease in the level of miRNAs, leading to the dysregulation of genes associated with the progression of pulmonary arteriopathy.

Recent advances in miRNA research

Recent improvements in miRNA studies [19,40,44-48] have helped us obtain additional information regarding the complicated role of these small RNA molecules in the development and progression of PAH associated with CHD. **Table 1** and **Figure 3** provides an overview of the

studies that investigated the role of miRNAs in PAH associated with CHD. Multiple studies [19,40,44-48] have demonstrated that in PAH associated with CHD, there is a dysregulation in the control of miRNAs, resulting in either a decrease or increase in their levels depending on the specific type of miRNA implicated. Alterations in miRNA expression are linked to various significant occurrences in PAH, such as cardiac hypertrophy/remodeling, angiogenesis, proliferation, and genes implicated with PAH pathobiology. Given the belief that PAH is a complex disease, identifying a singular cause responsible for its development will prove challenging.

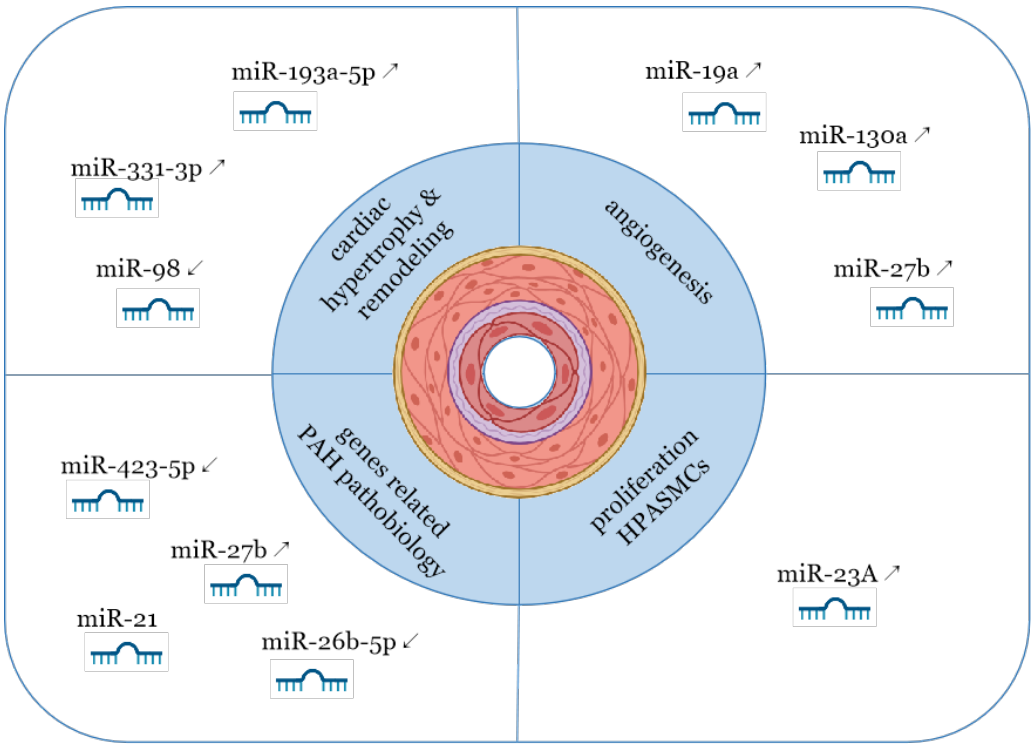

Figure 3. Recent finding in the roles of microRNAs in pulmonary arterial hypertension (PAH) associated with congenital heart disease (CHD). Multiple microRNA targets undergo alterations in expression and influence the development of PAD associated with CHD.

According to Ma *et al.*, miRNA profiling of the lungs of 12 patient with CHD (six of whom had PAH) showed that 62 miRNAs were significantly upregulated and 12 were significantly downregulated in the lung tissue of patients with PAH compared to non-PAH patients [44]. The study also discovered a significant correlation between the mean pulmonary arterial pressure before surgery and the levels of miRNA-27b expression, and an in vitro experiment showed that the overexpression of miRNA-27b reduced the expression of NOTCH1 protein [44]. Patients with CHD and PAH had lower levels of miR-98 than those with CHD and no PAH, and increased miR-98 expression was associated with a high cardiac index (CI). The same study also found that in patients with PAH associated with CHD, decreased miR-98 expression was linked to a high WHO grade of PAH severity. However, this investigation did not utilize the diagnostic gold standard examination (cardiac catheterization), to confirm PAH [45].

Elevated levels of miR-23a were observed in hypoxia-induced human pulmonary artery smooth muscle cells (HPASMC) and plasma samples obtained from individuals with PAH associated with CHD. The inhibitory effects of BMPR2 on the proliferation and migration of hypoxia-induced HPASMCs were negated by the overexpression of miR-23a, suggesting that these activities can be stopped by targeting miR-23a. miR-23a directly targets *BMPR2/Smad1* signaling. *BMPR2* is downregulated in PAH associated with CHD and hypoxia-induced HPASMCs. These findings improve our understanding of molecular pathways of the proliferation and migration of HPASMCs and suggest therapeutic alternatives for PAH associated with CHD [46].

Table 1. Summary of studies investigating the roles of microRNAs in pulmonary arterial hypertension (PAH) associated with congenital heart disease (CHD)

Author	Sample	Population characteristics	Participant age	Modality	MicroRNA target	Expression pattern	Potential action of microRNAs
Chouvarine *et al.* [48]	Plasma from cardiac catheterization	Twelve patients with PAH and nine non-PAH controls	3 months - 18 years old	qPCR array	miR-193a-5p; miR-423-5p; miR-26b-5p; miR-331-3p	Upregulated (miR-193a-5p and miR331-3p); downregulated (miR-423-5p and miR-26b-5p)	Some of these microRNAs are known to suppress genes that drive the pathobiology of PAH (miR-26b and miR-331) and to be involved in cardiac remodeling (miR-193 and miR-26b).
Zhang *et al.* [46]	Serum and cell cultures	Patients with PAH associated with CHD and healthy volunteers	34.8±7.2 years	RT-qPCR	miR-23A	Upregulated in the plasma of patients with PAH associated with CHD and hypoxia-induced HPASMCs	miR23a, part of the miR-23a/24/27a cluster on chromosome 19p13.12, is linked to several human diseases. Loss-of-function investigations showed that miR-23a knockdown inhibited HPASMC growth and migration under hypoxia. miR-23a may directly regulate BMPR2/Smad1 signaling in hypoxia-induced HPASMCs.
Zhang *et al.* [45]	Serum	Sixty cases of CHD were diagnosed using color echocardiography and the pulmonary artery systolic pressure was estimate accordance with the tricuspid regurgitation pressure	The CHD non-PAH group consisted of 15 individuals (8 males and 7 females) with a mean age of 4.96±1.93 years. The CHD-PAH group included 45 individuals (20 males and 25 females) with a mean age of 5.54±2.06 years.	RT-qPCR	miR-98	Downregulated	High levels of miR-98 were associated with high CI, and that patients with PAH associated with CHD had lower levels of miR-98 than patients with CHD and no PAH. miR-98 acts as a negative feedback mediator for many types of cardiac hypertrophy. The overexpression of miR-98 suppressed cardiac hypertrophy, hence regulating heart failure and pathological hypertrophy.
Chang *et al.* [40]	Blood	Patients with PAH caused by CHF, such as ASD and ventricular septal defect (VSD), and 10 healthy volunteers whose age and sex	Mean 51.67 years	RT-qPCR	miR-21	miR-21 has biphasic expression, increasing during the early phase (RV hypertrophy) and decreasing during the later phase (RV failure).	miR-21 was found to target genes involved in cardiomyocyte apoptosis, such as phosphatase and tensin homology deleted on chromosome 10 (*PTEN*) and Sprouty 2 (*SPRY2*)

Author	Sample	Population characteristics	Participant age	Modality	MicroRNA target	Expression pattern	Potential action of microRNAs
		were the same as those of the patients with PAH					
Tang [47]	Serum	Thirty patients with PAH associated with CHD and thirty healthy volunteers	20-80 years	RT-qPCR	miR-509-3p	Downregulated	-
Chen and Li [19]	Lung tissue and plasma	Six patients with PAH associated with VSD and six patients with VSD without PAH	PAH group: 4.0±1.2 years, control group: 3.8±0.9 years	microRNA arrays followed by RT-qPCR	miR-19a; miR-130a; miR-27b	Upregulated	miR-27b promotes angiogenesis by targeting antiangiogenic genes; miR-19a has a role in the vital activity of tumor angiogenesis and the proliferation of vascular smooth muscle cells; and miR-130a may have a permissive effect during angiogenesis.
Ma *et al.* [44]	Lung specimens	Six patients with PAH associated with VSD and six patients with VSD without PAH	1-7 years old	microRNA arrays followed by RT-qPCR	miR-27b	Upregulated	Acts in PAH via suppressing HPAECs' NOTCH1 expression.

Potential clinical applications of miRNAs in PAH-associated with CHD management

In addition to their role in the pathogenesis of PAH associated with CHD, miRNAs hold promise as potential biomarkers for diagnosis, prognosis, and therapeutic targets in the management of this disabling disease [18]. A study observed an upregulation of miR-424(322) expression in patients with PAH associated with CHD [42]. This increase in expression was found to be associated with both prognosis and disease severity [42]. In contrast, it is suggested that miR-424(322) functions by specifically targeting SMURF1 and regulating the BMPR2 pathway in cardiomyocytes, elevated levels of miR-424(322) were discovered to be associated with a more favorable prognosis [42].

Furthermore, another study showed that miR-509-3p expression was considerably lower in the PAH group than in the control group [47]. The value of the area under the curve (AUC) as a single factor is 0.694, indicating a modest diagnostic value. This can increase to 0.844 when combined with echocardiography examination, indicating that miR-509-3p has the potential as a biomarker [47]. By identifying specific miRNA patterns related to PAH associated with CHD, clinicians may be able to diagnose and prognosticate patients with increased accuracy as well as develop targeted therapies for improved treatment outcomes. The disease course can be decelerated with the help of a therapeutic approach that involves targets the appropriate miRNA [17]. However, it is imperative to emphasize the significance of having a substantial sample size in clinical studies. Additionally, it is crucial to conduct further investigations to ascertain the potential utility of miRNA as both an evaluation index and a biomarker for PAH associated with CHD.

Future directions and implications

While there is growing evidence of the involvement of miRNAs in PAH associated with CHD, further studies are still needed. Research regarding the biological influence of changes in miRNA expression in cardiac morphogenesis, particularly in CHD, is still urgently needed. Since many miRNAs are altered in expression levels during PAH associated with CHD, studying the interplay between them is also crucial. One possible direction for future studies is to investigate how miRNAs could be used as therapeutic agents. For example, synthetic miRNA mimics or inhibitors could be synthesized and administered to target specific miRNAs that are out of balance in PAH associated with CHD.

Conclusion

Recent advances in the study of miRNAs have provided important insights into the roles of these molecules in PAH associated with CHD. miRNAs play a crucial role in the pathogenesis of PAH associated with CHD by regulating various genes that are important for cardiac development. In addition, miRNAs have demonstrated promise as potential biomarkers for the diagnosis and prognosis of PAH associated with CHD. Furthermore, targeting specific miRNAs may hold promise as a therapeutic strategy for mitigating disease progression. In summary, recent advancements in comprehending the functions of miRNAs in PAH associated with CHD have provided insights into the fundamental mechanisms of this condition and have presented novel prospects for enhanced diagnostic, prognostic, and therapeutic interventions.

Ethics approval

Not required.

Acknowledgments

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

All data underlying the results are available as part of the article and no additional source data are required.

How to cite

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