

Invited Mini Review

Therapeutic implications of microRNAs in pulmonary arterial hypertension

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microRNAs (miRNAs) are a class of small, non-coding RNAs that play critical posttranscriptional regulatory roles typically through targeting of the 3'-untranslated region of messenger RNA (mRNA). Mature miRNAs are known to be involved in global cellular processes, such as differentiation, proliferation, apoptosis, and organogenesis, due to their capacity to target multiple mRNAs. Thus, imbalances in the expression and/or activity of miRNAs are involved in the pathogenesis of numerous diseases, including pulmonary arterial hypertension (PAH). PAH is a progressive disease characterized by vascular remodeling due to excessive proliferation of pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASCs). Recently, studies have evaluated the roles of miRNAs involved in the pathogenesis of PAH in these pulmonary vascular cells. This review provides an overview of recent discoveries on the role of miRNAs in the pathogenesis of PAH and discusses the potential for miRNAs as therapeutic targets and biomarkers of PAH. [BMB Reports 2014; 47(6): 311-317]

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a devastating and life-threatening disease, characterized by vasoconstriction of the pulmonary artery, resulting from the hyperproliferation of pulmonary vascular cells and consequent neointima formation in the small pulmonary arteries. This ultimately leads to right ventricular heart failure, which is the most common cause of death in PAH patients (1). Despite recent therapeutic advances, such as pulmonary vasodilators, survival rates still remain exceedingly low (2). In light of its progressive and lethal nature,

coupled with limited modalities of treatment, identifying novel therapeutic options via examining the signaling pathways involved in the pathogenesis of PAH remains a critical need.

microRNAs (miRNAs) are endogenous small non-coding RNAs that typically recognize the 3'-untranslated region (3'-UTR) of target messenger RNAs (mRNAs), predominantly resulting in inhibition of translation or destabilization of the target transcripts. They are expressed in a wide variety of organisms, from invertebrates to vertebrates, and are highly conserved between species (3). Approximately 2,000 miRNAs have been described in the human genome to date, which are estimated to target over 60% of human protein-encoding genes, indicating key roles in global cellular processes and the fine-tuning of gene expression in human cells (4, 5). Thus, physiological homeostasis is thought to be closely regulated by the balance of miRNA abundance, and deregulation of miRNA expression can lead to pathologic processes such as PAH. Indeed, an emerging body of evidence demonstrates that a fine balance in miRNA abundance is fundamental to maintaining homeostasis in the pulmonary vasculature, with miRNA imbalance playing an important role in the pathogenesis of PAH (6, 7). This review will discuss the function of miRNAs in well-defined signaling pathways involved in PAH pathogenesis.

miRNA BIOGENESIS AND FUNCTION

miRNAs are encoded in either intronic or exonic regions of protein-encoding genes, or intergenic regions of the genome, and over 40% of miRNAs originate in the genome in the form of polycistronic transcriptional units (8, 9). The biosynthesis of miRNA begins with generation of the primary miRNA (pri-miRNA) by RNA polymerase II; some miRNAs with upstream Alu sequences can be transcribed by RNA polymerase III (10). Then, the pri-miRNA is cleaved into the precursor-miRNA (pre-miRNA), a hairpin loop structure, through interaction with RNase III Drosha and its known cofactor DiGeorge Syndrome Critical Region 8 (DGCR8) in the nucleus. The pre-miRNA is then exported from the nucleus to the cytoplasm by exportin-5 and Ran-GTP. In the cytoplasm, the pre-miRNA is further processed by another RNase III, known as Dicer, resulting in the generation of a mature dou-

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ble-stranded miRNA consisting of the guide strand and passenger strand with a 5'-phosphate and two nucleotide 3' overhangs. Subsequently, only single-stranded mature miRNA can become incorporated into the RNA-induced silencing complex (RISC), association with which can guide the mature miRNA to target the 3'-UTR of mRNAs by imperfect base pairing (9, 11-13). Thus, a single miRNA can target tens to hundreds of mRNAs by imperfect miRNA-mRNA complementarity. Finally, this miRNA-mRNA association usually results in inhibition of translation or degradation of the target mRNA. Through fine-tuning the regulation of target transcripts, miRNAs play a critical role in maintaining the expression patterns of target genes and regulation of global cellular processes, including differentiation, proliferation, apoptosis, and developmental timing.

KEY miRNAs IN THE PATHOGENESIS OF PAH

From extensive studies, it has now been demonstrated that miRNAs are expressed in a cell- and tissue-specific manner and are crucially involved in numerous biological processes, with imbalances in their expression leading to various diseases. Emerging evidence indicates that miRNAs in the pulmonary vasculature play an important role in the maintenance of pulmonary vascular homeostasis and deregulation of these miRNA pathways is involved in the pathogenesis of PAH (7, 14, 15). PAH is a deadly disease associated with hyperproliferation of pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASCs), leading to structural changes in the pulmonary artery. The pathogenesis of PAH is complex, multifactorial, and largely unknown presently. However, dysfunction in PAECs and PASCs is closely involved in the pathogenesis of PAH and appears to play a major pathogenic role in mediating the remodeling of the pulmonary vasculature, increasing pulmonary vascular resistance, leading to right ventricular failure, and, ultimately, death (2, 16). Thus, much attention has recently been directed to these pulmonary vascular cells to determine the pathogenic mechanisms underlying PAH. These efforts have demonstrated that bone morphogenetic protein receptor type II (BMPR2), hypoxia, STAT3, and the apelin-APJ signaling pathway in pulmonary vascular cells are intimately involved in the pathogenesis of PAH (7, 15, 16). In the following sections, a discussion of the roles of miRNAs in PAH-related signaling pathways is provided.

The BMPR2 signaling pathway plays an essential role in the maintenance of pulmonary vascular homeostasis (17), and disruption of this pathway and/or genetic mutations in BMPR2 have been implicated in PAH (18, 19). Although BMPR2 dysfunction is considered a hallmark of the pathogenesis of PAH, few studies have assessed the mechanisms associated with BMPR2 dysfunction in PAH. In particular, there is a paucity of data concerning the involvement of miRNAs in these pathways. The first demonstration of a miRNA-BMPR2 axis in

PAH pathogenesis showed that IL-6 mediated activation of STAT3 resulted in inducing the expression of the miR-17-92 cluster in PAECs (20). Several miRNAs encoded by this cluster, specifically miR-17-5p and miR-20a, can target BMPR2 with consequential downregulation of BMPR2 protein expression (20). Based on this, two groups have evaluated the therapeutic potential of inhibitors of these two miRNAs (miR-17 and miR-20a) in PAH. Intravenous administration of a miR-17 inhibitor has been shown to ameliorate experimental pulmonary hypertension (PH) in rodent models. Additionally, the cyclin-dependent kinase inhibitor 1A (p21), a known target of miR-17, was increased in the lungs of mice and rats, as well as in human PASCs, following treatment with the miR-17 inhibitor (21). It has also been shown that treatment with the miR-20a inhibitor ameliorates right ventricular hypertrophy and pulmonary arterial vascular remodeling in the hypoxia-induced mouse model of PH, via induction of BMPR2 expression in lung tissues and activation of BMPR2 downstream signaling in PASCs (22). Importantly, miR-204 expression in PASCs is decreased in human PAH and in the monocrotaline (MCT)-induced rat model of PH. STAT3 activation was reported to lead to regulation of miR-204 in PASC (6). Indeed, STAT3 activation results in suppression of miR-204 expression, leading, in turn, to induction of Src kinase activity via upregulation of SHP2, a direct target of miR-204. This leads to subsequent hyperproliferation and resistance to apoptosis in PASC (6). Mutations in BMPR2 are predominant in most heritable cases of PAH, and mutations in its downstream mediator, the *SMAD9* gene (Smad8 protein), are also associated with heritable PAH (23). Drake *et al.* demonstrated that the *SMAD9* gene is essential for enhancing miRNA expression through non-canonical BMP signaling and mutation in this gene abrogates the induction of miR-21 and miR-27a expression completely. Consistently, the expression of miR-21 is decreased in PAECs and PASCs of patients with heritable PAH, leading to hyperproliferation of vascular cells (24). Thus, further studies are needed to examine the role(s) of BMPR2-related signaling in the pathogenesis of PAH, with a focus on modulating these miRNAs as a potentially attractive therapeutic option for PAH treatment.

Hypoxia is one of the hallmarks of PH and exposure to chronic hypoxia leads to PH pathogenesis via induction of PASC hyperproliferation and subsequent vascular remodeling. Given that chronic hypoxia is a key cause of PH, hypoxia-induced pulmonary vascular remodeling is a well-established animal model of PH (25). It has also been found that BMPR2 protein, but not BMPR2 mRNA, expression is downregulated by hypoxia, suggesting the involvement of miRNAs (26). Mizuno *et al.* showed that p53 knockout mice developed more severe PH, along with suppressed miR-34a expression, compared with wild-type mice, when exposed to chronic hypoxia. This study suggested that the hypoxia-p53-miR-34a axis may play an important role in hypoxic pulmonary arterial remodeling (27). It has been found that miR-145 is involved in

the pathogenesis of PAH and miR-145 expression was increased in wild-type mice exposed to hypoxia, as well as in PAH patients. Furthermore, remarkable protection against hypoxia-induced PH development was observed in miR-145 knockout mice and mice treated with a miR-145 inhibitor (28). A recent study investigated the effect of miR-190, which is induced by hypoxia and is expressed primarily in PASM of the hypoxic rat, on hypoxic pulmonary vasoconstriction. It was demonstrated that miR-190 overexpression resulted in significant vasoconstriction of the pulmonary artery through targeting of *Kcnq5* mRNA, which plays a major role in the regulation of membrane potential (29). Resistance of vascular cells to apoptosis is one of the key features of vascular remodeling associated with PAH and several studies have highlighted the role of miRNAs in this process. Indeed, miR-210 is a predominant hypoxia-induced miRNA and transcriptional induction of miR-210 by hypoxia-inducible factor-1a results in inhibition of PASM apoptosis via targeting of the transcription factor E2F3 (30). Hypoxia-induced miR-138 has also been shown to play an important role in apoptosis in PASCs. It was demonstrated that overexpression of miR-138 suppressed apoptosis of PASCs through targeting the pro-apoptotic serine/threonine kinase Mst1. This may suggest that miR-138 plays a key role in hypoxic pulmonary vascular remodeling (31).

Several studies have shown that miR-21 is regulated by BMPR2 and hypoxia signaling. This pathway is thought to be involved in the maintenance of pulmonary vasculature homeostasis and its dysfunction is associated with PAH (24, 32). Induction of miR-21 expression was shown in the lungs of mice exposed to hypoxia (21, 33), as well as in hypoxic PASCs (32, 33) and PAECs (34). miR-21 is also known to elicit anti-proliferative effects in both PAECs and PASC (24, 32, 33). Although a link between miR-21 and PAH has been identified, the function of miR-21 in PAH has been inconsistent in different experimental rodent models. Inhibition of miR21 expression by locked nucleic acid-modified anti-miR (33) or antagomiR (21) markedly ameliorated hypoxic PH through reduced systolic RVP and decreased muscularization of small distal pulmonary arteries (21, 33). In contrast, Parikh *et al.* showed that *miR-21* null mice developed more severe PH in response to SU5416 and chronic hypoxia (hypoxia/SU5416 model) when compared with wild-type control mice (34). It was also reported that expression of miR-21 was downregulated in the lungs of MCT-treated rats as well as in lung and serum samples from idiopathic PAH patients, whereas miR-21 expression was unaltered in samples from chronic hypoxia models (14). The inconsistencies between these studies may be due to differences in the species and/or experimental PH models used. Thus, further studies are needed to clarify the roles of miR-21 in PAH and to reveal the underlying mechanisms.

It has been demonstrated that the apelin-APJ pathway plays an important role in the maintenance of vasculature homeostasis. Disruption of apelin and its cognate G protein-coupled receptor APJ leads to significant deterioration of hypoxia-in-

duced PH in the mouse model, and apelin-APJ signaling is also disrupted in clinical PAH (7, 15). Recent studies designed to determine the downstream signaling targets of the apelin-APJ pathway have revealed two key miRNAs, miR-424 and miR-503, that target FGF2 and FGFR1 directly, promoting the pathogenesis of PAH. These miRNAs are downregulated markedly in PAECs isolated from patients with PAH and restoration of miR-424/503 expression in the hypoxia/SU5416 and MCT-induced experimental rat models contributes to amelioration of disease severity. This study confirmed that apelin-APJ signaling was involved in the maintenance of vascular homeostasis and showed that dysfunction in this pathway resulted in disruption of downstream signaling, which subsequently contributes to various diseases including PAH (7).

In addition to BMPR2, hypoxia, and apelin-APJ signaling-mediated miRNAs, it has been reported that other miRNA pathways also play important roles in the pathogenesis of PAH. Guo and colleagues showed that transgenic mice overexpressing miR-328 were at least partially protected against hypoxic PH. It is known that miR-328 targets the L-type calcium channel- α 1C and the insulin growth factor 1 receptor in PASM (35). It has been demonstrated that miR-206 is a tumor suppressor in various cancers (36, 37) and inhibition of miR-206 was shown to induce proliferation and reduce apoptosis in PASCs (38). Also, overexpression of miR-206 reversed these effects via targeting of Notch3, which is known to promote the development of PAH (38, 39). Recent studies have shown that miR-124 plays an important role in PASCs, as well as in pulmonary vascular fibroblasts, and may be involved in the pathogenesis of PAH. Through screening of miRNAs involved in the regulation of NFAT reporter activity, it was found that miR-124 targets multiple genes including NFATc1, CAMTA1, and PTBP1. This miRNA suppresses NFAT signaling through dephosphorylation, and consequently nuclear translocation of NFAT, leading to inhibition of PASM proliferation (40). Furthermore the role of miR-124 in pulmonary vascular fibroblasts was recently examined and it was shown that expression of miR-124 decreased markedly in fibroblasts isolated from both calves and patients with severe PH. Overexpression of miR-124 significantly reduced proliferation, migration, and monocyte chemotactic protein-1 expression in fibroblasts through targeting of polypyrimidine tract-binding protein 1. It was also found that miR-124 expression was downregulated by histone deacetylases and restoration of miR-124 by treatment with histone deacetylase inhibitors resulted in decreased proliferation and reduction of monocyte chemotactic protein-1 production in fibroblasts (41).

miRNAs AS POTENTIAL BIOMARKERS

It has been shown that some pre-miRNAs and mature miRNAs can be detected in the blood in a stable form, suggesting that beyond the intracellular roles of miRNA, extracellular miRNA

may play roles in the pulmonary vascular system. These extracellular miRNAs are released and protected by mechanisms including packing into exosomes, microvesicles, and associating with high-density lipoproteins or Argonaute2 (42-45). However, the exact mechanisms for release, the physiological significance, and the roles in pathogenesis of diseases remain unknown. Recent studies have reported that circulating miRNAs are associated with PAH, suggesting that these miRNAs could potentially serve as diagnostic biomarkers for PAH. One study showed that miR-21 was downregulated significantly in serum samples of patients with PAH (14). In addition, through microarray screening, it has been demonstrated that miR-150 expression is downregulated markedly not only in circulating microvesicles from PAH patients but also in the lungs of MCT-induced pulmonary hypertensive rats. Additionally, reduction of circulating miR-150 is associated with poor survival in PAH (46). In another study, a miRNA array used to examine the blood of patients with PH revealed that circulating miR-451 and miR1246 were downregulated in the buffy coats of these patients, whereas plasma levels of circulatory miR-23b, miR-130a, and miR-191 were markedly upregulated in the blood of PH patients, suggesting that these miRNAs may be useful as potential biomarkers for the early detection of the disease (47).

miRNAs as potential therapeutic targets in PAH

miRNAs that are able to target multiple protein-encoding genes play an important role in the maintenance of homeostatic balance in the pulmonary vasculature via regulation of global cellular processes, and disruption of miRNA expression may underlie the pathogenesis of various diseases including PAH. Thus, restoration of aberrant miRNA expression could have therapeutic value in the treatment of various diseases that are associated with abnormalities of miRNA expression. To restore miRNA expression to normal levels, two approaches have been used: anti-miRNA oligonucleotide (anti-miR)-based and miRNA mimic-based approaches (6, 21, 22). Although these miRNA-based therapies are an attractive strategy for the treatment of PAH, modulation of miRNA levels presents challenges in the clinical realm. First, administration of miRNA to the lung should be targeted to the vasculature or specific vascular cells, including PAECs, PSMCs, or fibroblasts, to minimize any off-target effects on other cells. Second, the dose of anti-miR or miRNA mimics delivered to the lung vasculature should be considered carefully again so that off-target effects are minimized. Thus, future research may focus on the development of vascular cell-specific delivery methods along with techniques that provide regulated miRNA release, such as modified nanoparticles. Alternatively, given that the goal of miRNA-based therapy is restoration of

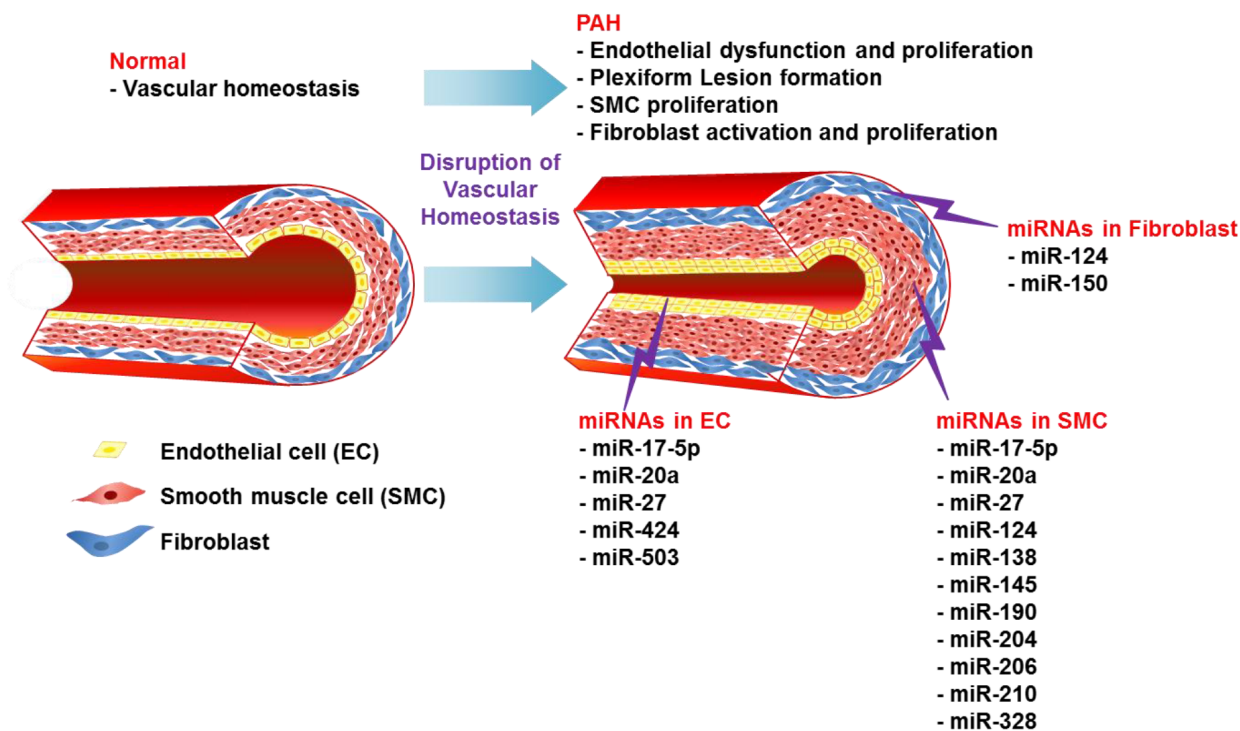


Fig. 1. Validated miRNAs in pulmonary arterial hypertension (PAH). Summary of PAH-related miRNAs in pulmonary vascular cells, including PAECs, PSMCs, and fibroblasts.

aberrant miRNA levels, development of small molecules that modulate the transcription factors or signaling pathways responsible for regulation of miRNA expression may also be an effective strategy.

CONCLUSIONS

miRNAs are key molecules in the maintenance of pulmonary vascular homeostasis. As described above, numerous studies support that dysregulation of various miRNAs in the pulmonary vasculature leads to abnormalities in protein-encoding gene expression and contributes to the pathogenesis of PAH (Fig. 1). Thus, the application of anti-miRs, miRNA mimics, and small molecules that are able to restore miRNA expression to physiological levels is an attractive therapeutic option in the treatment of PAH. Additionally, as aberrantly expressed miRNAs, including circulating miRNAs, are clearly correlated with specific diseases, they could prove to be useful biomarkers in disease diagnosis. Despite these advances in miRNA-PAH research, much work is still needed to better understand the network of miRNAs and their target mRNAs, involved in the pathogenesis of PAH.

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