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, PAH is a 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.

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 miRNAs. 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 (PASMCs). 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]

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 Rmase III Drosa and its known cofactor DiGeoroger 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 Rmase III, known as Dicer, resulting in the generation of a mature dou-
ble-stranded miRNA consisting of the guide strand and pas-
senger 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 pair-
ing (9, 11-13). Thus, a single miRNA can target tens to hun-
dreds of mRNAs by imperfect miRNA-mRNA complemen-
tarity. 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,
mRNAs play a critical role in maintaining the expression pat-
terns of target genes and regulation of global cellular proc-
esses, 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 pul-
monary 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 hyper-
proliferation of pulmonary artery endothelial cells (PAECs) and
pulmonary artery smooth muscle cells (PASMCs), leading to
structural changes in the pulmonary artery. The pathogenesis
of PAH is complex, multifactorial, and largely unknown
presently. However, dysfunction in PAECs and PASMCs 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 re-
sistance, 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), hy-
poxia, STAT3, and the apelin-APJ signaling pathway in pulmo-
nary 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 dis-
ruption of this pathway and/or genetic mutations in BMPR2
have been implicated in PAH (18, 19). Although BMPR2 dys-
function 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 in-
hibitor has been shown to ameliorate experimental pulmonary
hypertension (PH) in rodent models. Additionally, the cy-
clin-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 PASMCs, following treatment with the miR-17 in-
hibitor (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-in-
duced mouse model of PH, via induction of BMPR2 ex-
pression in lung tissues and activation of BMPR2 downstream
signaling in PASMCs (22). Importantly, miR-204 expression in
PASMCs is decreased in human PAH and in the monocrotaline
(MCT)-induced rat model of PH. STAT3 activation was re-
ported to lead to regulation of miR-204 in PASMC (6). Indeed,
STAT3 activation results in suppression of miR-204 expression,
leading, in turn, to induction of Src kinase activity via upregu-
lation of SHP2, a direct target of miR-204. This leads to sub-
sequent hyperproliferation and resistance to apoptosis in
PASMC (6). Mutations in BMPR2 are predominant in most her-
itable cases of PAH, and mutations in its downstream media-
tor, 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 abro-
gates the induction of miR-21 and miR-27a expression
completely. Consistently, the expression of miR-21 is de-
creased in PAECs and PASMCs of patients with heritable PAH,
leading to hyperproliferation of vascular cells (24). Thus, fur-
ther 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 ther-
apapeutic option for PAH treatment.

Hypoxia is one of the hallmarks of PH and exposure to
chronic hypoxia leads to PH pathogenesis via induction of
PASMC hyperproliferation and subsequent vascular remo-
deling. Given that chronic hypoxia is a key cause of PH, hypo-
oxia-induced pulmonary vascular remodeling is a well-estab-
lished animal model of PH (25). It has also been found that
BMPR2 protein, but not BMPR2 mRNA, expression is down-
regulated 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 C of the hypoxic rat, on hypoxic pulmonary vas oconstriction. It was demonstrated that miR-190 overexpression resulted in significant vas oconstric tion 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 C 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 PASC M C. It was demonstrated that overexpression of miR-138 suppressed apoptosis of PASM C 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 PASM C (32, 33) and PASE C (34). miR-21 is also known to elicit anti-proliferative effects in both PASE C and PASM C (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 miR-21 expression by locked nucleic acid-modified anti-miR (33) or antagoniR (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 SUS416 and chronic hypoxia (hypoxia/SUS416 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 vascular homeostasis. Disruption of apelin and its cognate G protein-coupled receptor APJ leads to significant deterioration of hypoxia-in-

**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, PASMCs, 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, PASMCs, and fibroblasts.
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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