Role of MicroRNAs in Hypoxia-Induced Pulmonary Hypertension
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Introduction
MicroRNAs (miRNAs) are small, non-coding RNA molecules (21–23nt) found in various species that play an important role in transcriptional and post-transcriptional regulation of gene expression [1]. miRNAs function by binding to complementary sequences within mRNA molecules, usually resulting in gene silencing via translational repression or target degradation [2,3]. The human genome may encode over 1000 miRNAs [4,5], which potentially target about 60% of mammalian genes [6,7]. Since the first miRNAs were characterized in the early 1990s [8], miRNAs have been found to be involved in many biological events including numerous diseases [9-12].

Chronic hypoxia is the most common cause of secondary pulmonary hypertension (PH) for which the mechanisms are still largely unknown [13-15]. Recent studies have implicated miRNAs for having an important role in hypoxia-induced responses in various cellular processes including cell apoptosis and proliferation [16,17]. In addition, the role of miRNAs in chronic hypoxia-induced pulmonary vascular remodeling and pulmonary hypertension has been characterized.

miRNAs in the Regulation of Calcium Influx and Channels
A rise in cytosolic Ca\(^{2+}\) concentration in pulmonary arterial smooth muscle cells (PASMCs) is an important stimulus for pulmonary vasoconstriction and vascular remodeling. Increased resting cytosolic Ca\(^{2+}\) and enhanced Ca\(^{2+}\) influx have been observed in PASMCs from patients with idiopathic pulmonary arterial hypertension (IPAH). Li et al. [18] reported that hypoxia resulted in a significant increase of the miR-190 levels in the pulmonary artery. In addition, PASMCs treated with synthetic miR-190 had similar changes in pulmonary arterial vasoconstriction and Ca\(^{2+}\) influx compared to hypoxia-exposed pulmonary arteries. Furthermore, synthetic miR-190 remarkably enhanced the vasoconstrictive responses to phenylephrine and KCl. These effects were caused by the targeting of voltage-gated K+ channel subfamily member Kcnq5 mRNA by miR-190. Moreover, antagomiR-190 was found to partially reverse the effects of miR-190 on PASMCs and pulmonary arteries. These results suggest that increased miR-190 by hypoxia enhances Ca\(^{2+}\) influx, therefore leading to pulmonary vascular constriction.

Another study by Guo et al. [19] showed that miRNA-328 was drastically decreased in the pulmonary artery after a hypoxic exposure. Transgenic mice over-expressing miR-328 had similar changes in pulmonary vascular resistance in rodents and accounted for the proliferative and antiapoptotic phenotypes of PAH-PASMCs. Therefore, reduction of miR-204 levels promotes PASMC proliferation and resistance to apoptosis. On the other hand, increasing miR-204 levels in PAH-PASMCs reverses the pro-proliferation and antiapoptotic phenotype of PAH-PASMCs. Src activation by miR-204 inhibited NFAT activation in PAH-PASMCs, and the miR-204–Src–STAT3–NFAT axis was activated in the PAH animal models, suggesting that miR-204 is critical to the etiology of PAH. In addition, Guo et al. [19] had reported that miRNA-328 suppressed insulin-like growth factor 1 receptor, ultimately leading to the apoptosis of PASMCs.

Jalali et al. have demonstrated that miR-206 regulates PASMC proliferation and differentiation [23], miR-206 expression in mouse PASMCs was correlated with an increase in right ventricular systolic pressure. Reduction of miR-206 levels in human PASMCs caused increased proliferation and reduced apoptosis, and these effects were reversed by the over-expression of miR-206. miR-206 over-expression also increased the levels of smooth muscle cell differentiation markers

miRNAs in the Regulation of PASMC Proliferation and Apoptosis
Pulmonary hypertension is associated with abnormal smooth muscle cell proliferation and antiapoptosis [14,20]. A number of studies have been undertaken to determine the role of miRNAs in smooth muscle cell proliferation and apoptosis. Puillamsetti et al. [21] reported that inhibition of miR-17 caused an up-regulation in cyclin-dependent kinase inhibitor 1A (p21), a potent cell cycle inhibitor. An in vitro study demonstrated that over-expression of miR-17 significantly reduced p21 expression and increased the proliferation of smooth muscle cells. In both a chronic hypoxia-induced PH mouse model and a monocrotaline-induced PH rat model, inhibition of miR-17 improved heart and lung function by interfering with pulmonary vascular and right ventricular remodeling. These beneficial effects are likely attributed to the up-regulation of cell cycle-related genes such as p21.

A separate study by Courboulin et al. [22] demonstrated that miR-204 expression in PASMCs is down-regulated in both human and rodent pulmonary arterial hypertension (PAH) lungs compared to normotensive lung samples and that down-regulated miR-204 levels were specific to the lung in rats with PH. miR-204 down-regulation in the lungs correlated directly with PAH severity as measured by pulmonary vascular resistance in humans and mean pulmonary artery pressure in rodents and accounted for the proliferative and antiapoptotic phenotypes of PAH-PASMCs. Therefore, reduction of miR-204 levels promotes PASMC proliferation and resistance to apoptosis. On the other hand, increasing miR-204 levels in PAH-PASMCs reverses the pro-proliferation and antiapoptotic phenotype of PAH-PASMCs. Src activation by miR-204 promoted STAT3 and NFAT activation in PAH-PASMCs, and the miR-204–Src–STAT3–NFAT axis was activated in the PAH animal models, suggesting that miR-204 is critical to the etiology of PAH. In addition, Guo et al. [19] had reported that miRNA-328 suppressed insulin-like growth factor 1 receptor, ultimately leading to the apoptosis of PASMCs.

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α-smooth muscle actin and calponin, illustrating its importance in the regulation of differentiation of PASMCs.

miRNAs in the Regulation of BMPR2 Signaling

Deficiency of bone morphogenetic protein receptor type 2 (BMPR2) signaling is a hallmark feature that has been described in pulmonary hypertension [20,24-27]. In addition to genetic regulation of BMPR2 signaling, recent studies have indicated that miRNAs also play an important role in the modulation of BMPR2 signaling. Brock et al. [28] reported that interleukin-6 (IL-6) modulates the expression of the BMPR2 through a STAT3–miRNA cluster 17/92 pathway. Ectopic over-expression of miR-17/92 resulted in a strong reduction of the BMPR2 protein, and BMPR2 was shown to be directly targeted by miR-17-5p and miR-20a. Knockdown experiments have demonstrated that IL-6 regulates miR-17-92 through STAT3. Consistent with these data, they found a highly conserved STAT3 binding site in the promoter region of the miR-17/92 gene. Finally, they showed that persistent activation of STAT3 led to repressed protein expression of BMPR2. Other experiments [29] were recently performed to identify the role of miR-20a as a regulator of the expression of BMPR2 in pulmonary arterial vascular remodeling. In vitro data showed that introduction of antagonmiR-20a in human PASMC resulted in activation of downstream targets of BMPR2 (Id1 and Id2) with reduced proliferation. In addition, treatment with antagonmiR-20a enhanced the expression levels of BMPR2 in lung tissues. Moreover, antagonmiR-20a significantly reduced wall thickness and luminal occlusion of small pulmonary arteries and reduced right ventricular hypertrophy due to hypoxia, suggesting the beneficial role of specific inhibition of miR-20a in PH development and that restoration of functional levels of BMPR2 in pulmonary arteries can be achieved by miRNAs.

miR-145 and miR-21 have also been demonstrated to be involved in the regulation of BMPR2 pathway. Increased expression of miR-145 in mice exposed to hypoxia as well as in lung tissue of patients with idiopathic and heritable PAH was reported by Caruso et al. [30]. Elevated levels of miR-145 expression were found in primary PASMCs isolated from patients with BMPR2 mutations and also in the lungs of BMPR2-deficient mice. miR-145 deficiency in miR-145 knockout mice and mice treated with antagonmiR-145 conferred pulmonary protective functions from the development of PAH.

In addition, aberrantly expressed miR-21 has also been described in the contribution to altered BMPR2 signaling and PH development [31,32]. Elevated expression levels of miR-21 in vivo were observed in pulmonary tissue from several rodent models of PH and in humans with PH. Sequestration of miR-21 diminished chronic hypoxia-induced PH and attenuated hypoxia-induced pulmonary vascular remodeling. Over-expression of miR-21 enhanced the proliferation of human PASMCs in vitro, whereas down-regulation of miR-21 diminished PASMC proliferation. Hypoxia and BMPR2 signaling up-regulated miR-21 expression independently in pulmonary arterial endothelial cells. In a reciprocal feedback loop, miR-21 down-regulated BMPR2 expression.

Circulated miRNAs

Recent studies have shown that miRNAs are circulating freely in the mammalian blood and can be used as predictive biomarkers for early diagnosis [33]. Rhodes et al. recently reported that reduced miRNA-150 is associated with poor survival in PAH [34]. Plasma miR-150 levels correlated with 2-year survival in patients with PAH. Cox regression analysis also confirmed miR-150 levels as a significant predictor of survival. In a multivariable model, plasma miR-150 levels were verified as an independent predictor of survival in PAH. In addition, Wei et al. [17] identified several dysregulated miRNAs (miR-451, miR-1246, miR-23b, miR-130a, and miR-191) in the circulation of PH subjects, and the levels of these miRNAs were proportional to the degree of PH. Therefore, these miRNAs may be considered as potential biomarkers for early detection of PH.

Concluding Remarks

Growing evidence indicates that miRNA regulation is involved in many biological events and processes and is one of the key mechanisms for mediating cellular responses under various stress conditions. A number of studies support the idea that deregulation of miRNA expression leading to abnormal target gene expression contributes to the pathogenesis of pulmonary diseases. Therefore, understanding the molecular mechanisms underlying miRNA-modulated target genes will lead to a development of more effective therapies for patients with PH.

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