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Vascular Remodeling in Hypoxia-induced Pulmonary Hypertension: Role of Cytokines and MicroRNAs

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Vascular remodeling is the most important factor in the pathogenesis of pulmonary hypertension and is mainly driven by changes in the expression of the bone morphogenetic protein receptor type II (BMPR2) that controls the growth of pulmonary arterial endothelial cells and vascular smooth muscle cells. Mutations in the BMPR2 are identified in about 70% of familial and in up to one third of idiopathic pulmonary arterial hypertension. Reduced expression of BMPR2 however have also been identified in non-genetic forms of the disease, for example in hypoxia-induced pulmonary hypertension. This dysregulation might be due to the action of microRNAs (miRs), which are small RNA fragments that bind the complementary mRNA of BMPR2 and result in posttranscriptional gene silencing. Here, we review the most important pathways identified in the context of miRs and hypoxia-induced pulmonary hypertension and emphasize the potential of miRs as targets for future causative therapies.

Keywords: Bone morphogenetic protein receptor type II, hypoxia-induced pulmonary hypertension, interleukin-6, microRNAs, vascular remodeling

DEFINITION

Pulmonary hypertension is hemodynamically defined as an elevation of the mean pressure in the pulmonary arteries above 25 mmHg, which, clinically, results in dyspnea, reduced exercise capacity, fainting and progredient right heart failure. Pulmonary arterial hypertension (PAH) is considered to be a rare disease with prevalence of 15-50/millions. PAH is due to hereditary changes in distinct genes or is associated with other conditions such as infections with the human immunodeficiency virus and schistosomes, cirrhotic liver disease, hyperthyroidism, rheumatologic disorders, medication with anorexigens and congenital heart disease. Pulmonary hypertension in its wider sense; however, includes elevations of the mean pulmonary arterial pressure due to left-sided cardiac disease and hypoxic disorders as well as states of pulmonary hypertension following chronic thromboembolic events and miscellaneous other, mostly rare conditions.^[1] It is evident that the whole group of entities that might result in pulmonary hypertension is more than being an orphan disease, of major clinical importance. In particular, pulmonary hypertension associated with hypoxia is commonly encountered in patients with chronic obstructive

pulmonary disease (COPD), interstitial lung disease, sleep-disordered breathing and alveolar hypoventilation. Moreover, the mechanisms of hypoxia to provoke elevations in the pulmonary circulation are employed experimentally in one of the established animal models of the disease, i.e., the hypoxia-induced pulmonary hypertension in rodents.

The pathophysiology of almost all forms of pulmonary hypertension is characterized by a triad of vasoconstriction, microthrombosis and vascular remodeling. In hypoxic conditions, due to a reflex known by the eponym Euler-Liljestrand, which improves ventilation/perfusion mismatch by limiting blood flow to non-ventilated areas of the lung, hypoxic vasoconstriction is probably the most important early mediator of pulmonary hypertension. Rarefaction of the pulmonary vessels by emphysematous destruction of the lung parenchyma, alveolar hypoventilation and inflammation further aggravate both hypoxia and pulmonary hypertension.^[2] Vascular remodeling, finally, defined by the thickening of small pulmonary arteries due to the proliferation of vascular smooth muscle cells and formation of a neointima that occludes the vascular lumen by transmigration of these cells from the medial to the endothelial layer, results in permanent elevation of pulmonary pressure.

INTERLEUKIN-6

One of the most important inflammatory cytokines associated with pulmonary hypertension and chronic obstructive lung disease is IL-6. IL-6 is produced by inflammatory cells, i.e., monocytes and T-lymphocytes and as such, a major mediator of the hepatic acute-phase response. Cultured pulmonary arterial smooth muscle cells, however, were also found to increase the production of IL-6 when exposed to hypoxia.^[3] Elevated levels of IL-6 have been described in the circulation of patients with pulmonary hypertension.^[3-5]

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In PAH, these levels could be correlated with survival and were found to be better predictors of a bad outcome than traditional prognostic markers (i.e., 6 min walking distance, hemodynamic values).^[6] In COPD patients, levels of IL-6 were found to be a good discriminator for the presence of pulmonary hypertension.^[3] In addition, several animal models have investigated the impact of IL-6 on pulmonary pressure: subcutaneous injection of IL-6 increased pulmonary arterial pressure in rats, an effect found to be enhanced by hypoxia.^[7] Overexpression of IL-6 showed right ventricular hypertrophy and angioproliferative lesions in the pulmonary arterial vessels in mice under normoxic conditions and further accentuated under hypoxic conditions,^[8] whereas animals deficient of IL-6 were found to show less cardiac and vascular sequelae of pulmonary hypertension when exposed to hypoxia.^[9] *In vitro*, the addition of IL-6 to pulmonary arterial endothelial cells was found to increase the levels of microRNAs (miRs) derived from the poly-cistronic miR-17/92 cluster: A pathway, which results in reduction of the expression of the bone morphogenetic protein receptor type II (BMPR2),^[10] the most important mediator of pulmonary vascular remodeling.

BMPR2 is a member of the transforming growth factor beta receptor (TGFBR) family expressed on vascular endothelial and smooth muscle cells, which, upon binding of its ligands, heterodimerizes with receptor type I activating a signaling pathway that promotes a pro-apoptotic and anti-proliferative phenotype. Mutations of the gene encoding for BMPR2 are found in about 70% of familial and in 11-40% of idiopathic PAH (referred as hereditary PAH).^[11-14] Asymptomatic carriers of BMPR2 mutations have recently been found to show an increased vascular response when exposed to hypoxic pressure.^[15] Animals with heterozygous mutations in BMPR2 developed more severe pulmonary hypertension under hypoxic conditions, probably due to endothelial dysfunction, than the wild-type controls.^[16] Dysregulations of BMPR2, in particular downregulation without respective mutations, have also been described in other, non-genetic forms of pulmonary hypertension^[17] and in the two most established experimental models mimicking the human disease (i.e., the monocrotaline model^[18] and the hypoxia-induced model of pulmonary hypertension^[19]). Moreover, targeted gene delivery restoring BMPR2 has proven successful in treatment of experimental pulmonary hypertension.^[20] These data highlight the utmost importance of BMPR2 for pulmonary vascular remodeling, whether present in the form of genetic mutations that might lead to symptomatic disease following hypoxia (or other second hits) or due to non-genetic downregulation upon hypoxic conditions.

miRS

The downregulation of BMPR2 in experimental pulmonary hypertension was found to be mediated by a phylogenetically conserved pathway involving the IL-6 regulated transcription factor signal transducer and activator of transcription (STAT) 3 and miRs.^[10,21] MiRs are non-coding RNA fragments consisting of 20-22 nucleotides that, following intracellular maturation and cleavage involving the action of the RNase III enzymes Drosha and Dicer, bind by Watson Crick base pairing to complementary sites within the mRNA sequence of a target gene. Since this interaction prevents further translation of the bound mRNA, either by direct inhibition or, alternatively, by

degradation of the target mRNA, miRs are associated with gene silencing. In hypoxia-induced pulmonary hypertension, the miR cluster miR-17/92 (hosting six different miRs, i.e., miR-17, miR-18a, miR-19a, miR-19b, miR-20a, miR-92a) is of special interest. Upon binding of IL-6 to vascular endothelial cells, STAT3 is activated by phosphorylation, which, in turn, induces the expression of miR-17/92. miR-20a and similarly but to a somewhat lesser extent, the functionally related miR-17 directly bind to the 3' untranslated region of the mRNA of BMPR2 thus inhibiting its translation into protein.^[22,23] The elevated levels of IL-6 observed in patients with COPD thus might be directly associated with the reduced expression levels of the BMPR2 protein in hypoxia-induced pulmonary hypertension. Elevated levels of IL-6, however, have also been observed in PAH patients and conversely, constitutive activation of STAT3 has been described in plexiform lesions isolated from the vessels of patients with PAH.^[24] The miR-BMPR2-pathway thus might be of major importance also in pulmonary hypertension unrelated to hypoxia or in the absence of elevated IL-6 levels. In hypoxia-induced pulmonary hypertension these data, however, might have direct implications for the therapeutic management: first, IL-6 can be inhibited specifically by a novel biological drug (tocilizumab, F. Hoffmann-La Roche AG[®]) and second, miRs have been successfully targeted with antagonomiRs.^[25] We are not aware of data blocking IL-6 in pulmonary hypertension with the exception of anecdotal case reports;^[26,27] however, experimental *in vivo* results obtained from the use of antagonomiRs are promising regarding further development.

AntagonomiRs, as suggested by their name, are specific antagonists of miRs. In experimental pulmonary hypertension, antagonomiRs directed against miR-17^[28] and miR-20a^[21] have been injected intravenously and intraperitoneally in rodents and were found to restore functional expression levels of BMPR2, to improve the pulmonary hemodynamics and to prevent or even reverse pulmonary vascular remodeling. The safety and tolerance of such approach in patients is currently investigated for antagonizing miR-122 by miravirsin (SPC3649, Santaris[®]) in patients chronically infected with hepatitis C virus in a phase 2 study.

Several other miRs have been identified to be upregulated or downregulated in pulmonary (arterial) hypertension [Table 1 and Figure 1]. In response to hypoxia, for example, miR-210 was found to be upregulated by hypoxia-inducible factor-1a (HIF)-1a directly targeting an apoptotic transcription factor (E2F3).^[29] However, the functional relevance of most alterations, in particular in hypoxia-induced elevations of the pulmonary pressure, remains to be elucidated. Similarly, data focusing on other miR-targets than BMPR2 are rare and due to the overwhelming importance of BMPR2, might play a minor role in pulmonary hypertension. The other genetic mutation identified for the development of PAH, activin receptor-like kinase 1, is a TGFBR type I and has not been subjected to miR-mediated control of expression. Conversely, the TGFBR type II has been described to be downregulated by the action of miR-130a, which has also been found upregulated in hypoxia-induced pulmonary hypertension (Brock *et al.*, unpublished work). Since the interactions between these receptors are redundant and appear to be cell-type specific with different downstream signaling events in endothelial and smooth muscle cells,^[30] the pathogenetic impact of miR-130a remains unclear and is currently investigated.

Table 1: miRs in pulmonary hypertension					
miR	Expression	Trigger	Target	Reference	
miR-17	Upregulated	IL-6 STAT3	BMPR2	Brock <i>et al.</i> 2009 ^[10] Pullamsetti <i>et al.</i> ^[28]	
miR-20a	Upregulated	IL-6 STAT3	BMPR2	Brock <i>et al.</i> 2009 ^[10] Brock <i>et al.</i> 2012 ^[21]	
miR-204	Downregulated	STAT3	SHP2	Courboulin <i>et al.</i> ^[31]	
miR-21	Upregulated	?	BMPR2	Yang <i>et al.</i> ^[32]	
miR-145	Upregulated	BMPR2 deficiency	?	Caruso <i>et al.</i> ^[33]	
miR-150	Down regulated	Associated with poor survival	?	Rhodes <i>et al.</i> ^[34]	
miR-210	Upregulated	HIF-1α	E2F3	Gou 2012 <i>et al.</i> ^[29]	

miR = MicroRNA; STAT = Signal transducer and activator of transcription; BMPR2 = Bone morphogenetic protein receptor type II; HIF = Hypoxia-inducible factor; IL-6 = Interleukin-6

Figure 1: Hypoxia and miR-mediated vascular remodeling. Exposure to hypoxia induces the expression of IL-6, which, following binding to the respective surface receptor on endothelial cells, activates STAT3. STAT3 drives the expression of miR-17/92 host gene C13orf25. miR-17 and miR-20a target the mRNA of BMPR2, finally disrupting the downstream signaling pathway, including the activation of Smad transcription factors and the respective Smad target genes, e.g., inhibitor of differentiation and cyclin-dependent kinase inhibitor 1a (also known as the tumor suppressor p21). By repressing miR-204, STAT3 induces the tyrosine phosphatase SHP2 and the nuclear factor of activated T-cells. miR-210 is upregulated by the HIF-1α and targets the transcription factor E2F3. All pathways result in an anti-apoptotic and pro-proliferative phenotype

PERSPECTIVES

Since the association between miRs and pulmonary hypertension was initially suggested, miRs have gained growing scientific interest. The most established role of miRs focuses on the pathogenesis of pulmonary hypertension. Commonly acting as post-transcriptional gene silencers, miRs alter the expression of anti-proliferative target genes (e.g., the cell surface receptor BMPR2) in endothelial and smooth muscle cells and in turn promote vascular remodeling of small pulmonary arteries. The use of miRs as biomarkers for diagnosis or prognosis is currently studied. The project, finally, to employ miRs as targets for a potentially causative treatment to reverse vascular remodeling is not yet beyond an experimental stage but, together with the impact of miRs in pathogenesis and the role of miRs as prognostic markers, emphasizes the interesting field of translational research in pulmonary hypertension.

DISCLAIMER

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