**Pulmonary arterial hypertension: molecular genetic basis and emerging treatments**

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**Abstract**

Pulmonary arterial hypertension (PAH) is a rare cardiovascular disorder caused by narrowing of blood vessels in the lung and in the absence of therapy leads to right heart failure and death. No cure for this devastating disorder is known. The major objective of the current treatments is to improve symptoms and these therapies were developed prior to the discovery that this disease has substantial genetic components. In this review, we discuss molecular genetic basis of PAH together with pathobiology, current and future therapeutic interventions.

**Introduction**

Pulmonary arterial hypertension (PAH) is a rare cardiovascular disorder characterised by the sustained elevation of blood pressure in pulmonary arteries, recorded above 25 mm of Hg during rest and 30 mm of Hg during exercise[1]. The rise in the pressure occurs due to occlusion of pulmonary arteries in the lungs leading to subsequent right heart failure and death, if left untreated. It is estimated that approximately 146,000 subjects suffer from this devastating disease across the EU, USA and Japan (see www.leaddiscovery.co.uk/reports/424/PulmonaryArterial_Hypertension). Although the cases of PAH have been reported in India, Pakistan and Bangladesh yet a lack of epidemiology accounts for the unavailability of PAH patients in the Indian subcontinent[2].

The underlying mechanisms of the pathogenesis of PAH remain unknown. However, many investigations support the role of a cell surface receptor, bone morphogenetic

protein receptor 2 (*BMPR2*) in development and progression of PAH[3], as germline mutations have been identified in the majority of patients with a family history. Moreover, PAH has been shown to occur in correlation with other pathological condition such, scleroderma, portal hypertension and hereditary *haemorrhagic telangiectasia* and infection of HIV[4], HHV[5], Hepatitis Virus B and C[6-7]. Environmental factors such as hypoxia[8] and chemical like monocrotaline[9], appetite suppressor drugs anorexigens[10] and nerve cell stimulants like methamphetamine and cocaine[11] have also been shown to play a role in the disease pathogenesis. Monocrotaline and hypoxia have been used to induce the disease in model organisms including mouse and rat.

PAH is caused by the obstruction of pulmonary arteries due to excessive proliferation of cells in *tunica intima*, *tunica media* and *tunica adventitia* along with deposition of components of extracellular matrix. The formation of occlusive lesion initiates in the *tunica media*, called as medial thickening, as the pulmonary arterial smooth muscle cells (PASMCs) start to proliferate. Medial thickening is followed by the proliferation of endothelial cells and transitional cells in the *tunica intima* and fibroblasts in the *tunica adventitia*. Intimal thickening occurs in two phases, initially myofibroblast like cells, originating from the PASMCs, migrate to the endothelial lining and start dividing and then the collagen deposition occurs. However, in the most severe occlusions, plexiform lesions are formed by the proliferation of endothelial cells and multiple capillary channels. The thickening in *adventitia* occurs due to proliferation of fibroblast cells that are sensitive to stimuli like hypoxia, monocrotaline treatment and high perfusion blood flow.

In this review we aim to understand the molecular basis of current therapies and how the genetic basis of PAH is likely to change the conventional therapeutic approaches.

**Molecular basis of current therapies**

The current therapies for PAH are based primarily on vasoeffectors, a set of molecules that controls the blood flow in the vasculature. They could either dilate the blood vessels by relaxing the PASMCs or constrict the blood vessels by contracting them. The former are vasodilators and the latter are referred as vasoconstrictors. They also control the proliferation of PASMCs and activation of platelets. Human body maintains a physiological balance between the dilators and constrictors and thus the imbalance plays vital role in PAH pathogenesis. (review in [12]). Almost all the vasoeffector molecules have been shown at aberrant levels in PAH (Table 1).

Since, understanding of the disease pathogenesis was based on vasoeffector molecules, the first drug for PAH, approved in 1996, was a prostacyclin analogue - Epoprostenol[13]. Many drugs followed that targeted particular vasoeffector molecules, particularly NO, serotonin and endothelin (Table 2). Depending upon the role of the vasoeffector, the compound aimed at increasing or decreasing its activity by targeting the vasoeffector molecule itself, its receptors or the synthesis pathways. For example, most of the endothelin inhibitors target the endothelin receptor and the NO based drugs aim at inhibiting the PDE-5 receptors and increasing the level of cGMP and increasing NO bioavailability by targeting the synthesis of the enzyme endothelial nitric oxide synthase – eNOS. Sildenafil Citrate, sold under trade name REVATIO by Pfizer, reduces degradation of cGMP by inhibiting PDE-5[14].

Though the approach discussed above appears to be simpler, but the compounds in question only improve the symptoms. They do not target the underlying cause of PAH. Most of these compounds have shown side effects including nausea, headache and oedema. Some of these drugs have shown to affect the metabolism of the liver. It has been shown that bosentan increased the level of liver enzymes, aspartate aminotransferase or alanine aminotransferase[15]. Similarly, Sitaxsentan has been withdrawn by Pfizer due to the adverse side effects on pregnancy and impairment of lactations[16].

**Molecular genetic basis of PAH**

Heterozygous mutation in the *BMPR*2 gene underlies the majority of the inherited and familial forms of PAH. Mutations are also found in SMAD genes namely SMAD1, 4 and 9 but they represent an infrequent cause of the disease[17]. Dysfunctional BMPR-II or SMAD-mediated signalling potentiates the TGF-beta signalling. Increasing number of studies suggest the involvement of TGFβ signaling in PAH. *In vitro*, TGF-β1 elicits a pro-proliferative response in PASMCs isolated from PAH patients (PAH-PASMCs) compared to controls [18]. Elevated TGFβ signaling has been implicated in a number of preclinical PAH models, including the aorto-pulmonary shunt model in lambs [19], hypoxia-induced PAH in mice and the monocrotaline (MCT)-induced model in rats [20].

The TGFβ family of ligands convey signalling through a complex of two cell surface receptors, type-I and type-II. On binding the appropriate ligand, the type-I receptor (also known as ALK) phosphorylates the type-II receptor. Subsequently, the type-II receptor phosphorylates a set of cytoplasmic proteins known as regulatory SMAD (R-SMADs).

Activated R-SMADs form a complex with co-SMAD and translocates to the nucleus, where they regulate transcription.

Both, TGF-β and BMP mediated signalling are instrumental for the maintenance of cellular homeostasis. They are involved in differentiation and development and control both proliferation and differentiation of PASMCs and endothelial cells. Although, nonsense mutations in the *BMPR2* gene have been discovered as the major genetic determinant in familial PAH[21], yet the cause of the aberration in TGF-β signalling is not well understood. A linking protein called TGF-β associated kinase 1 (TAK1) has been proposed as one of the key determinant as inhibition of this protein inhibited excessive PASMC proliferation [22]. TAK1 seems to be interacting with both BMP and TGF-β receptors and in the event of *BMPR*2 mutation identified in PAH patients, the BMP receptor is not able to take as much as TAK1 as the wild type receptor. This makes TAK1 accessible to TGF-β receptor which elicits pro-proliferative activity.

**Therapeutic resolution based on the genetics of PAH**

Although gene therapy has been proposed for the treatment of many genetic disorders, yet development of suitable vectors remains a big challenge. Most of these vectors are virus based and the subjects need to be immune-compromised and hence this route is far from achieving routine clinical success.

Antibiotic compounds such as aminoglycosides and several small molecule compounds, including oxadiazole compound PTC124[23], acetyl-amino benzoic acid derivatives[24] and nitrobenzene compounds RTC13/14[25], have proposed as a therapeutic strategy for the treatment of nonsense associated genetic diseases including cystic fibrosis and

Duchene muscular dystrophy as these compounds have been shown to promote translation readthrough of nonsense alleles. Promotion of readthrough in PAH-related cell-based studies [26-27] suggests that agents that promote translation readthrough of *BMPR*2 nonsense alleles may be therapeutically beneficial.

Another strategy for the resolution of PAH appears to be the inhibition of TGF-β signalling. Inhibition of TGF-β signalling in both monocrotaline and hypoxia induced animal models by known inhibitor of ALK-5 has reduced excessive PASMC proliferation and also reversed muscularisation of pulmonary arteries[28]. However, targeting this pathway carries substantial risk due to its role in maintaining normal homeostasis. Therefore, the inhibition of this pathway requires a deeper understanding of TGF-β signaling and the context in which it acts, as well as the cellular consequences of its inhibition.

## Conclusion

Until recently, PAH has been considered to be a highly progressive disorder, resulting death in many cases. However, the impact of *BMPR2* gene mutations and the involvement of the aberrant TGF-β signalling in the disease pathogenesis has opened new avenues for developing therapies for this devastating disease. Drugs that inhibit the overactive TGF-β signalling or resolve the biochemical consequences of *BMPR2* gene defects could potentially provide protection prior to or following the onset of PAH.

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