Targeting the TGF-β signalling pathway for resolution of Pulmonary Arterial Hypertension (PAH)

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Abstract

Aberrant TGF-β signalling activation is linked to pulmonary arterial hypertension. *BMPR2* gene mutations perturbs the balance between BMP and TGF-β pathways, leading to vascular remodelling, narrowing of the lumen of pulmonary vasculature, and clinical symptoms. This forum highlights the association of the TGFβ pathway with pathogenesis and therapeutic approaches.

What is Pulmonary Arterial Hypertension (PAH)?

Sustained elevation of the pulmonary arterial pressure (PAP) above 25mm Hg at rest or 30mm Hg during exercise with a normal pulmonary capillary wedge pressure (≤15mm Hg) in the absence of underlying heart, lung or thrombo-occlusive disorders is clinically known as pulmonary arterial hypertension (PAH). The thickening of the pulmonary arterial wall and luminal obliteration of the small peripheral arteries observed in the PAH patient's lungs lead to the elevated vascular resistance and PAP. In an attempt to maintain the pulmonary circulation against the soaring afterload, the right ventricle works harder which eventually weakens the heart muscle and in the absence of effective therapy, right heart failure and death ensue [1].

It is estimated that approximately 146,000 subjects suffer from pulmonary hypertension across the EU, USA, and Japan. Furthermore, there are number of poorly characterised cases that are diagnosed late in the disease progression, creating significant room for growth for development of novel therapies [1].

Current therapies for PAH are very costly with an estimated annual treatment and care expenditure for each PAH patient varying substantially between medications, with £39,000 for iloprost, £23,500 for bosentan, £6,000 for sildenafil and £120,000 for treprostinil with combination therapy potentially reaching an average of a £200-300,000 per year[i]. In addition, the mean annual resource used per PAH patient in the UK is, 86.1 prescription claims, 24.6 outpatient visits, 20.3 GP visits, 6.2 inpatient admissions, and 0.9 AE visits and an average of £28,000 for an eight-day in-patient stay [2].

Genetic basis of PAH

Heterozygous germline mutations in the bone morphogenetic protein type II (BMPRII) receptor encoding the *BMPR2* gene, underlie the majority (>80%) of the heritable and familial PAH cases. BMPRII is a member of the transforming growth factor β (TGF-β) superfamily of receptors. Upon ligand stimulation, the receptor complex generates a phosphorylation relay of cytoplasmic proteins, including the SMAD family capable of transducing the downstream signalling pathways. BMPs signal through a specific set of regulatory SMADs namely SMAD 1, 5 and 9, whilst TGF-β-mediated signalling occurs via SMAD2/3 proteins **(Figure 1)**. However, a reduced penetrance in the family members suggests the involvement of a 'second hit' in the precipitation of the disease besides mutations within the *BMPR2*. Additionally, mutations in SMAD family members namely SMAD 1, 4 and 9 and other TGF-β superfamily members including *ALK1, ENG*, *TGFBRII, BMPR1B* and *BMP9* have been identified. Mutations in each of these genes disrupt the BMP4/9/BMPR-II-induced SMAD1/5 pathway leading to the loss of the growth suppressive effects of BMPs on vascular cells [3]. Additionally, an enhanced resistance to apoptosis and excessive proliferation in response to BMP (e.g. 2, 4 and 7) and TGFβ (e.g. TGF-β1) ligand stimulation have been observed in PASMCs deficient in BMPRII receptor, which are likely to contribute in narrowing the pulmonary vascular lumen **(Figure 1)**. Furthermore, mutations have also been identified in *TBX4, EIF2KA4, ATP13A3, AQP1* and *SOX17* genes, but they represent an infrequent cause of the disease.

Over activation of TGFβ pathway in PAH

We and others have demonstrated that BMPRII deficiency leads to an abnormal and overactivation of the TGF-β1 signalling pathway [4,5] **(Figure 1)**. The TGFβ pathway is activated both in PAH patients [9] and in several pre-clinical PAH models, including: aorta-pulmonary shunt model in lambs, chronic hypoxia-induced PAH in mice, the rat monocrotaline (MCT) models [4, 6], or infection with schistosomiasis.

Mechanistic data from these animal models have shown similar evidence of elevated TGF-β1 ligand expression [6] and downstream transcriptional activity [4,6]. Moreover, enhanced TGF-β signalling in these models has been associated with PAH accompanied by smooth muscle hypertrophy, perivascular fibrosis, and extracellular matrix remodelling [1]. TGF-β1 elicits a pro-proliferative response in PASMCs isolated from PAH patients (PAH-PASMCs) compared to controls [5,7]. Pulmonary arterial endothelial cells (PAECs) expressing the mutant BMPRII receptor, secrete an elevated level of TGF-β1 ligand which further accelerates the proliferation of PASMCs [8].

In an attempt to elucidate the mechanistic intersection between BMP and TGF-β pathways, we have demonstrated that in the event of mutations in BMPRII receptor, TGFβ- associated kinase 1 (TAK1), the regulator of Mitogen Activated Protein Kinase (MAPK) pathways becomes more accessible to TGF-β pathway, which activates the p38MAPK signalling aiding the pro-proliferative response of the mutant PASMCs [9]. Attenuation of TAK1 through TAK1-specific inhibitor, oxozeanol inhibits proliferation and induces apoptosis in *BMPR2* mutant mouse PASMCs [9].

These observations advocate that a dysregulated TGF-β signalling accelerates the remodelling of pulmonary vasculature and thus plays a pathogenic role in clinical and pre-clinical PAH cases, suggesting that targeting of this pathway may have therapeutic benefits in PAH.

BMP signalling as a therapeutic target

The indispensable role of BMPRII during embryogenesis and development is demonstrated by the findings that, mice with homozygous null mutation for this protein is lethal before gastrulation. However, BMPRII deficient mice (*bmpr*$2^{+/-}$) can survive normally and breed although they exhibit enhanced susceptibility to develop PAH, right ventricular hypertrophy and remodelling of peripheral pulmonary arteries either spontaneously at the age of six months or upon exposure to inflammatory stress, hypoxia or stimulation by serotonin. These observations suggest the need of a 'second-hit' for triggering the disease predisposition and may explain the reduced penetrance of the disease in BMPRII mutation carriers. PASMCs isolated from these transgenic mice models showed increased TGF-β1 ligand expression and downstream transcriptional and splicing activities [4,9,10]. In a rat model, adenovirus-mediated *BMPR*2 gene therapy reduced the pulmonary hypertensive response to chronic hypoxia. Selective enhancement of BMPRII either by administration of BMP9 ligand [10] or FK506 reverses established PAH in these mouse and rat models [11]. However, enhancement of the BMPRII by means of promotion of translation readthrough using chemical agents such as PTC124 failed to restore BMPRII-mediated defects [12].

Developmental status of current approaches for the resolution of PAH

The above-mentioned observations suggest that agents which either promote BMPRII mediated signalling or inhibit the overactive TGF-β signalling or restore the balance between these two

signalling pathways may provide protection prior to or the following the onset of PAH. In this section, we are outlining the developmental status of current approaches for therapeutic intervention of PAH.

BMPR2 gene therapy

Targeted adenovirus mediated delivery of wild type BMPRII to the pulmonary endothelium of monocrotaline (MCT) and hypoxic rat models have been successful in ameliorating the PAH associated pulmonary haemodynamic parameters and regressing the remodelling of the pulmonary vasculature [13, 14]. Importantly, a noticeable downregulation of TGF-β expression has been observed in the BMPRII reinstated lung sections and pulmonary endothelium of MCT induced PAH rats [14]. In this experimental model, activation of the attenuated BMPRII/SMAD 1/5 pathway is observed while the SMAD 2/3 and p38 MAPK signalling are downregulated which further supports the mechanistic paradigm on how PAH may develop due to the imbalance in BMPRII and TGF-β signalling pathways [14,15].

However, vector toxicity, cell specificity, reliability for sustained delivery of the target gene, stimulation of host immunogenic response, insertion of unwanted and oncogenic mutations upon integration and effect of the over-expression of BMPRII on other downstream signalling cascades are some hurdles that may limit this approach being translated clinically [16]. Nonetheless, the irrefutable therapeutic benefits of *BMPR*2 gene therapy on preclinical PAH models demand this technology to be advanced by careful consideration of all its shortcomings in the context of PAH treatment.

Translation readthrough

Translation readthrough agents such as aminoglycosides, PTC124 and RTC13/14 have been proposed for the resolution of PAH, which elicited beneficial effects in other nonsense-associated genetic disorders such as cystic fibrosis and Duchene Muscular Dystrophy. We have tested translation readthrough drugs including PTC124 and aminoglycosides, but they failed to restore BMPR-II-mediated signalling. Furthermore, we have found that aminoglycosides such as gentamicin, G418 sulfate, tobramycin promoted translation readthrough in cell-based assays [12]. Until to date, these translation readthrough agents have not been tested in pre-clinical PAH animal models. Additionally, the risk of renal and otic toxicity of aminoglycosides at high doses and the need for intramuscular administration may limit their success in routine clinical application in PAH.

BMP signalling modulators

Promotion of BMP signalling using small molecule agents including prostacyclin analogues such as iloprost, treprostinil and PDE5 inhibitor such as sildenafil have been shown to rescue the activation of BMP/SMAD1/5 axis, enhance the expression of *Id*1 transcripts and restore the growth suppressive effects of the BMP ligands in PASMCs harbouring a *BMPR*2 mutation [17,18]. These agents have reversed the progression of established PAH and vascular remodelling in MCT-induced rats through cAMP/protein kinase A (PKA)-dependent and cGMP/cGKI-dependent fashion, respectively [17,18]. While prostacyclin analogues and PDE5 inhibitors are approved in PAH treatment, they are associated with significant cost, short half-life, instability, catheter related-infection and devoid of remedial function.

Moreover, FDA approved immunosuppressant FK506 (tacrolimus) potentiated SMAD1/5 dependent BMPRII signalling in patient derived PAECs through a dual mechanism of action by i) binding to the BMP repressor FK-binding protein-12 (FKBP12) and dislodging it from the type I receptor, and ii) by acting as an inhibitor of phosphatase calcineurin. This compound has successfully inhibited the development of established disease in multiple preclinical PAH models including *BMPR*2 knock-in mice, MCT induced and sugen 5416-hypoxic rats [11]. Although low-dose FK506 has been declared safe, in Phase IIa clinical trials it has failed the 6-minute walk test. This could be due to a small heterogenous sample size, incomplete follow-up and the requirement to monitor drug specific biomarkers [19].

The beneficial effects of the BMP9 ligand have been reported in 2015 which secured the monolayer barrier integrity *in vitro*, while in experimental models it prevented and reversed the disease suggesting its therapeutic potential in PAH [10]. However, the ligand named as MGX292 is yet to be tested in Phase I-II clinical trials.

Selective inhibition of the TGFβ pathway shows beneficial effects in PAH animal models

Inhibition of the overactive TGFβ signalling by ALK5 inhibitors including IN1233, SD208 and SB525334, biologics like Sotatercept (Acceleron) and combination therapy such as pirfenidone with sildenafil have shown beneficial effects in pre-clinical PAH animal models as reflected by reductions in PAP, Right ventricular hypertrophy (RVH) and the muscularization of distal pulmonary arteries [6, 7, 9].

Sotatercept (ACTRIIA-Fc), a recombinant fusion protein combining the extracellular domain of the human activin receptor type IIA (Act-RII) to the Fc domain of human IgG1 has been tested in cell-based and pre-clinical animal models. In cell based assays, this selective TGF-β ligand trap has attenuated the SMAD2/3 activation, restored the BMP9-BMPRII pathway and maintained the vascular homeostasis [20] In animal models, this agent has altered the remodelled pulmonary vasculature and attenuated the PAH severity [20]. Primary results from PULSAR and SPECTRA trials[ii, iii]have demonstrated success in improving the vital endpoints of PAH including the 6-min walk test leading to multiple Phase III trials such as STELLAR trial[iv] .

Furthermore, selective inhibition of TGF-β signalling using recombinant TGFBRII receptor (TGFBRII-Fc), has attenuated RVH and reduced pulmonary hypertension in hypoxia, sugen-hypoxia and MCT-induced animal models. Previously, we have shown that treprostinil, a prostacyclin analogue mitigate the overactivated TGF-β signalling pathways by downregulating the expression of phospho-SMAD3 protein, and inhibit the progression of PAH in the MCT-induced PAH rat model [4]. Another prostacyclin analogue beraprost abrogates the abnormal proliferation of mice primary PASMCs harbouring a *BMPR*2 mutation in the presence or absence of TGF-β1 stimulation. Taken together, these vasodilators reduce the pulmonary arterial pressure by restricting the overactive TGF-β signalling [4] while favouring the BMPRII/SMAD 1/5 axis through the cAMP/PKA-dependent pathway [16].

Limitations and prospects of current therapies

The fact that the complexity associated with selective targeting of Type I or Type II TGFβ receptors and induction of untoward cellular responses create a major hurdle for the development of kinase domain inhibitors of TGF-β receptors. An alternative approach is to target the extracellular domain

of the TGF-β receptors which, unlike kinase domain inhibitors, are likely to produce a temporo-spatial shortage of TGF-β and hence minimise the issues of inadequate selectivity and cell permeability. However, delivering the proteinaceous agents including the ligands or ligand traps through the most acceptable oral route may impose a challenge to the formulation scientists.

While developing such inhibitors, patient stratification needs to be considered. Both preventive and curative treatment options should be available for PAH patients with and without BMPRII mutation carriers who are at high risk of developing the disease. The drugs should be sufficiently stable and have shelf life of more than two years. The current management therapies are expensive and not well suited for widespread use in low and middle-income countries and therefore new approaches should be cost-effective. The agents should not lead to contraindications or adverse effects (e.g. local pain or infection at infusion site). Oral delivery is preferred due to high patient acceptance and ease of administration, and the interventions should have considerably long half-life to avoid frequent administration (e.g. beraprost have a half-life of only 35-40 minutes).

Concluding remarks

Aberrant TGF-β signalling has been implicated in the pathogenesis of PAH and restricting this pathway offers an attractive treatment option. Most of the TGF-β targeted therapeutics demonstrate improvements in rescuing PAH disease phenotype in experimental animal models and clinical trials. This suggests that regulating either TGF-β or BMP signalling may be effective in reversing or preventing PAH progression. However, a combinational therapy that elicits anti-TGF-β and pro-BMP activities may have better therapeutic benefits than a single treatment alone. Tissue specific approaches focusing on the pulmonary vasculature may also alleviate the untoward effects associated with non-specific targeting.

Figure 1

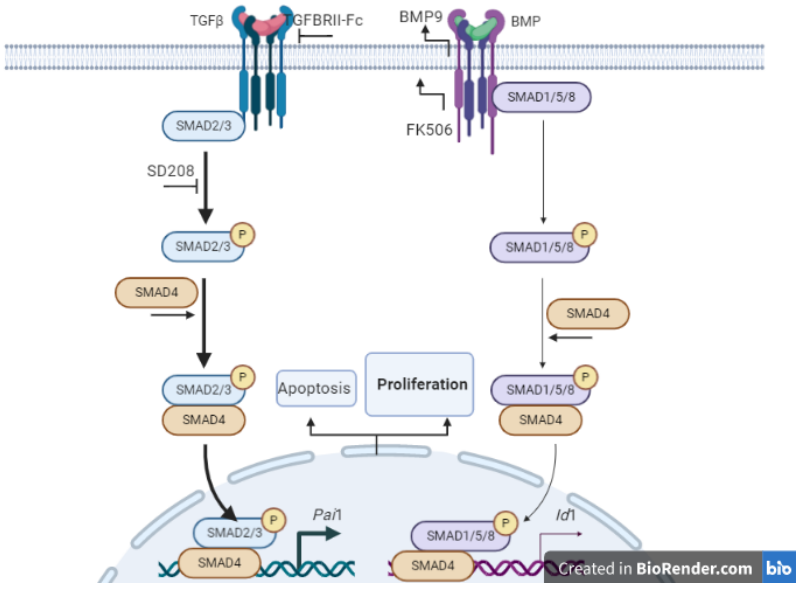

Figure.1 TGFβ signalling pathway in PAH. In PAH, the dysfunctional BMPR-II-mediated signalling impairs the SMAD1/5/8 pathway (right), whilst the overactive TGF-β pathway results in the dysregulated SMAD2/3 pathway (left) leading to abnormal transcriptional activation of target genes (e.g. *Id*1 for BMP and *Pai*1 for TGF-β) leading to excessive proliferation and attenuation of apoptosis. Selected inhibition and activation of specific cellular activities in the BMP and TGF-β signalling pathways have been indicated by activation and inhibitor arrows, respectively. The thickness of the arrows indicates the strength of the signalling cascades. Created in Biorender.com.

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Resources

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[ii]https://clinicaltrials.gov/ct2/show/NCT03496207

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