Pathobiology of pulmonary arterial hypertension: understanding the roads less travelled

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ABSTRACT   The pathobiology of pulmonary arterial hypertension (PAH) is complex and incompletely understood. Although three pathogenic pathways have been relatively well characterised, it is widely accepted that dysfunction in a multitude of other cellular processes is likely to play a critical role in driving the development of PAH. Currently available therapies, which all target one of the three well-characterised pathways, provide significant benefits for patients; however, PAH remains a progressive and ultimately fatal disease. The development of drugs to target alternative pathogenic pathways is, therefore, an attractive proposition and one that may complement existing treatment regimens to improve outcomes for patients. Considerable research has been undertaken to identify the role of the less well-understood pathways and in this review we will highlight some of the key discoveries and the potential for utility as therapeutic targets.

Introduction

Pulmonary arterial hypertension (PAH) is a progressive and ultimately fatal disease of the pulmonary vasculature. Over the past three decades, there have been major advances in our understanding of the pathobiology of PAH, leading to the development of targeted therapies and improved patient outcomes [1].

The clinical manifestations of PAH result from a reduction in the cross-sectional area of the pulmonary arteries [1], driven by a combination of vasoconstriction, thrombosis, inflammation, and proliferative and obstructive remodelling of the pulmonary artery wall [2]. Like all arteries, the pulmonary artery wall is composed of an outer layer of connective tissue, extracellular matrix (ECM), nerve cells and fibroblasts, and a secondary layer consisting of vascular smooth muscle cells (SMCs) or smooth muscle-like pericytes. Finally, there is a single layer of endothelial cells (ECs), which are in contact with circulating blood [3–5]. The SMCs provide structural integrity and play a crucial role in controlling the diameter of the vessel in
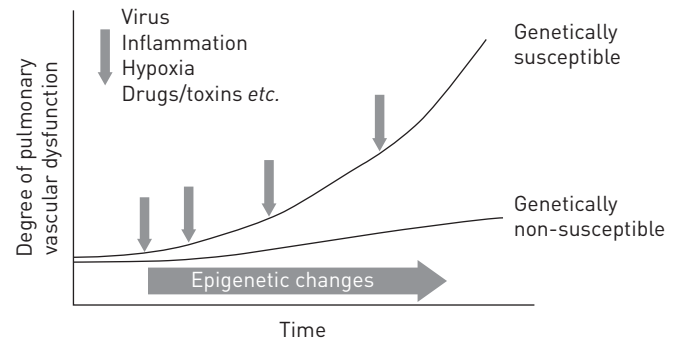
FIGURE 1 A genetic predisposition combined with environmental risk factors may be involved in the development of pulmonary arterial hypertension (PAH). In individuals without mutations in PAH-associated genes (genetically non-susceptible), epigenetic changes and exposure to environmental triggers over time can result in progressive pulmonary vascular dysfunction. This process is accelerated in individuals carrying mutations in PAH-associated genes (genetically susceptible). Reproduced and modified from [8] with permission from the publisher.

response to stimuli [6]. Communication between ECs and SMCs is important for homeostasis within the pulmonary circulation, and can regulate vascular tone and vessel diameter [3–5].

At a molecular level, the pathogenic pathways that drive the development of PAH are still not completely understood. All currently approved PAH therapies target one of three relatively well-characterised pathways (nitric oxide, endothelin and prostacyclin) [1]. However, a multitude of other molecular pathways have been implicated in the pathogenesis of PAH [7], and a genetic predisposition combined with environmental risk factors may be involved in the initial stages of the disease (figure 1) [2]. Intensive research efforts are ongoing to elucidate the complex pathogenic mechanisms that underlie this devastating disease, and this topic will be discussed extensively at the World Symposium on Pulmonary Hypertension in 2018.

The focus of this article is to discuss current research on some of the less well-characterised pathways, from the structural and mechanical changes in the ECM associated with vascular stiffness, to the modification of pulmonary arterial pressure associated with iron deficiency (figure 2, table 1). This article concentrates on pathways of our own particular interest. However, we recognise that there is a wealth of data providing insights into a multitude of other pathogenic pathways in PAH, beyond those covered in this review. Some of these pathways are highlighted in table 2, but are not discussed further in this article. In a timely and relevant review, the metabolic theory of PAH is reviewed comprehensively in the article by CHAN and RUBIN [91] in this issue of the European Respiratory Review.

Increasing our understanding of the pathogenic processes underlying the development of PAH provides an opportunity to identify novel therapeutic targets and to expand and improve the treatment options that are available for PAH. Novel therapeutic agents currently in clinical development have recently been reviewed by SIMONNEAU et al. [45] in the European Respiratory Review. In this article, we have highlighted a number of molecular targets that may be of interest for future clinical development (tables 1 and 2).

Vascular stiffness

Vascular stiffening has been associated with significant morbidity and mortality in a number of vascular diseases [92]. The mechanisms involved include increased wall thickness, SMC proliferation, and changes in the type and quantity of ECM proteins [92]. ECM modifications occur early in the development of PAH and involve matrix degradation and remodelling, as well as changes in the balance between collagen and elastin deposition [9].

Two recent studies by BERTERO and co-workers [9, 10] have provided insights into the molecular pathways involved in vascular stiffening in PAH, and have indicated a role for the co-transcription factors YAP/TAZ (Yes-associated protein/transcriptional coactivator with PDZ-binding motif) and the microRNA-130/301 family (miR-130/301). In 2015, BERTERO et al. [9] demonstrated that ECM remodelling activates YAP/TAZ in pulmonary vascular cells. This induces expression of miR-130/301, which leads to additional ECM remodelling and further up-regulation of YAP/TAZ in a positive feedback loop. A potential pathogenic role for this pathway in pulmonary hypertension (PH) is further supported by observations that inhibition of miR-130/301 is associated with decreased severity of PH in a rat model [9]. In their subsequent study, a relationship between vascular stiffness, YAP/TAZ and metabolic pathways was identified in experimental PH in rats. Specifically, ECM stiffening, via YAP/TAZ activation, increases the rate of glutaminolysis and production of aspartate, which is required for continued cellular proliferation and migration in stiffened
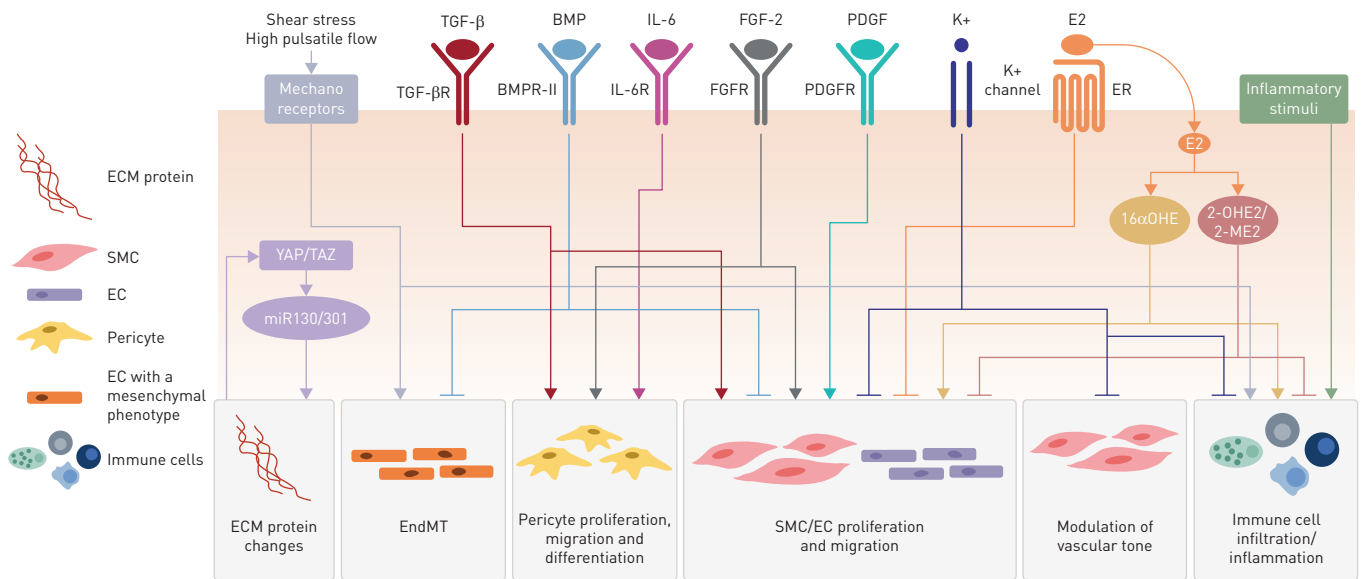
FIGURE 2 Diverse cellular processes and signalling pathways contribute to the pathogenesis of pulmonary arterial hypertension (PAH). Dysfunction in a myriad of overlapping signalling pathways can promote endothelial cell (EC) proliferation and differentiation, smooth muscle cell (SMC) proliferation, migration and vasoconstriction, pericyte proliferation, migration and differentiation, endothelial-to-mesenchymal transition, immune cell infiltration and extracellular matrix (ECM) remodelling in the pulmonary artery. This figure illustrates the pathways and processes discussed in this article and is not an exhaustive illustration of all the pathways currently understood to be involved in the pathogenesis of PAH. EndMT: endothelial-to-mesenchymal transition; TGF-β: transforming growth factor- β; TGF-βR: TGF-β receptor; BMP: bone morphogenetic protein; BMPR-II: BMP receptor type 2; IL-6: interleukin-6; IL-6R: IL-6 receptor; FGF-2: fibroblast growth factor-2; FGFR: FGF receptor; PDGF: platelet-derived growth factor; PDGFR: PDGF receptor; E2: oestradiol; ER: oestrogen receptor; YAP/TAZ: Yes-associated protein/transcriptional coactivator with PDZ-binding motif; 16αOHE: 16α-hydroxyoestrone; 2-OHE2: 2-hydroxyoestradiol; 2-ME2: 2-methoxyoestradiol; miR130/301: microRNA-130/301 family.

ECM [10]. These studies support the idea that the molecular pathways activated in response to pulmonary vascular stiffening might be a target for novel therapies in PAH.

Endothelial-to-mesenchymal transition

Severe PAH is associated with the formation of neo-intimal vascular lesions in the pulmonary arteries, caused by increased proliferation of ECs and SMCs. The formation of this neo-intima may involve the process of endothelial-to-mesenchymal transition (EndMT) [11]. In EndMT, ECs acquire a mesenchymal phenotype, a gene expression profile more commonly observed in SMCs, and develop the ability to migrate and remodel the ECM [11, 12]. A role for EndMT in the development of PAH is supported by the presence of cells with a mixed mesenchymal/endothelial phenotype in intimal lesions from PAH patients, alongside identification of RNA and protein signatures associated with EndMT [13].

Mechanical changes associated with PAH, including high shear stress and high pulsatile flow [12], can be detected by mechanoreceptors on the surface of ECs, leading to expression of proliferative genes [3, 14, 15] and potentially triggering EndMT [16]. At a molecular level, transforming growth factor (TGF)-β has been implicated in the induction of EndMT [17, 18], with a reduction in TGF-β signalling being associated with reduced neo-intima formation and decreased contribution of endothelial-derived cells to the neo-intima *in vivo* [17]. Recently, HOPPER *et al.* [11] have provided insights into the mechanism by which TGF-β can induce EndMT. They demonstrated, in pulmonary ECs, that dysfunctional signalling through bone morphogenetic protein receptor type 2 (BMPR-II), a member of the TGF-β receptor superfamily, leads to increased expression of High Mobility Group AT-Hook 1, which binds to DNA and may promote binding of pro-EndMT transcription factors [11].

There is growing evidence that EndMT occurs in the development of human PAH. Targeting the pathways involved in this process may provide an opportunity to develop anti-remodelling therapies [12].

Pericyte-mediated vascular remodelling

Pericytes are involved in the stabilisation, maturation and remodelling of vessels, as well as in the regulation of vascular tone and proliferation of ECs [19, 93]. Abnormal endothelial–pericyte cross-talk can contribute to vascular remodelling [20], and a role for pericytes in the pathogenesis of PAH is supported

TABLE 1 Pathogenic pathways and potential therapeutic targets in pulmonary arterial hypertension (PAH)

Pathway	Role in PAH	Potential therapeutic targets	Refs
Vascular stiffness	Can activate the YAP/TAZ co-transcription factors, leading to further ECM remodelling and modulation of metabolic pathways	Glutaminase YAP/TAZ	[9, 10]
Endothelial-to-mesenchymal transition	Can be induced by haemodynamic changes associated with PAH TGF-β signalling and HMGA1 may play a role in EndMT EndMT cells can migrate, remodel the ECM and have increased apoptosis resistance	HMGA1 TGF-β BMPR-II	[11–18]
Pericyte-mediated vascular remodelling	Increased pericyte density in distal pulmonary arteries has been reported in PAH FGF-2 and IL-6 can stimulate pericyte migration and proliferation TGF-β can promote the differentiation of pericytes into SMCs	FGF-2 IL-6 TGF-β	[19, 20]
TGF-β signalling	Loss of function heterozygous *BMPR2* mutations have been reported in PAH In the absence of a mutation, BMPR-II expression is frequently reduced in PAH Suppression of BMPR-II signalling leads to increased proliferation and decreased apoptosis in vascular cells Inflammatory mediators, *e.g.* TNF-α, may play a role in PAH pathogenesis in the context of *BMPR2* mutations	BMPR-II TNF-α SMURF 1 miR-140-5p	[21–32]
PDGF and FGF signalling	Over-expression of PDGF and FGF has been reported in PAH and may be involved in abnormal proliferation and migration of SMCs, as well as endothelial dysfunction	FGF-2 PDGF	[33–44]
Inflammation and immunity	Inflammatory mediators and cell infiltrates are frequently observed in PAH Vascular cells can respond to inflammatory stimuli by enhanced proliferation and migration and reduced apoptosis	CD20 B IL-1β and IL-6 TNF-α	[45–58]
Resting membrane potential	Loss-of-function *KCNK3* mutations have been reported in PAH May contribute to pulmonary vasoconstriction and pulmonary vascular remodelling Expression of the Kv1.5 channel is also reduced in human and experimental PAH	KCNK3 Kv1.5	[59–67]
Oestrogen signalling	E2 metabolites can exert both detrimental and protective effects in PAH E2 may directly protect against the development of PH in animal models	miR-29 E2 metabolites	[68–75]
Iron homeostasis	Iron deficiency may play a role in pulmonary vascular remodelling	Iron replacement	[76–81]

TGF-β: transforming growth factor-β; PDGF: platelet-derived growth factor; FGF: fibroblast growth factor; YAP: Yes-associated protein; TAZ: transcriptional coactivator with PDZ-binding motif; ECM: extracellular matrix; HMGA1: High Mobility Group AT-Hook 1; EndMT: endothelial-to-mesenchymal transition; BMPR-II: bone morphogenic protein receptor type 2; IL: interleukin; SMC: smooth muscle cell; TNF-α: tumour necrosis factor-α; SMURF: SMAD-specific E3 ubiquitin protein ligase; miR: microRNA; CD20 B: cluster of differentiation 20 B-lymphocyte antigen; KCNK3: potassium channel subfamily K member 3; Kv1.5: voltage-dependent potassium channel 1.5; E2: oestradiol; PH: pulmonary hypertension.

by the observation of a two-fold increase in pericyte number in distal pulmonary arteries from the lungs of PAH patients compared with controls [19]. This study also demonstrated that pulmonary EC-derived fibroblast growth factor (FGF)-2 and interleukin-6 can stimulate pericyte migration and proliferation, and that overexpression of TGF-β in isolated PAH lung tissue promotes the differentiation of pericytes into SMC-like cells. These responses may potentially contribute to pulmonary vascular remodelling [19]. Therapies targeting the molecular pathways involved in pericyte biology may therefore be potential options for the treatment of PAH.

Growth factor signalling
TGF-β signalling
Patients with heritable PAH (HPAH) frequently carry heterozygous mutations in genes encoding receptors of the TGF-β superfamily, most commonly *BMPR2*, which encodes the cell surface receptor BMPR-II [21].

TABLE 2 Additional pathogenic pathways and potential therapeutic targets in pulmonary arterial hypertension (PAH)

Pathway	Role in PAH	Potential therapeutic targets	Refs
Transcription factors	FoxO1, a member of the Forkhead box O (FoxO) family of transcription factors that are key regulators of cellular proliferation, is downregulated in pulmonary vessels/PASMCs of human/experimental PH lungs Activation of the prosurvival transcription factors STAT3, NFAT, and HIF-1α has been demonstrated in experimental models of PAH	FoxO1 STAT3/PIM1 NFAT, HIF-1α	[28, 82, 83]
NOTCH3-HES5 signalling	*NOTCH3* is overexpressed in small PASMCs in human PH There is evidence for a link between NOTCH3 receptor signalling through HES5 and SMC proliferation in the development of PAH	NOTCH3-HES5 pathway	[84]
Epigenetic mechanisms	Methylation-induced downregulation of SOD2 in PASMCs may create a metabolic state that favours proliferation and suppresses apoptosis Aberrant expression of HDACs and BRD4 (a transcriptional regulator that recognises acetylated lysine residues) is consistent with altered epigenetic mechanisms in PAH Studies have shown that miRNAs (small RNA molecules that negatively regulate expression of target genes) are dysregulated in patients with PAH	SOD2 HDACs BRD4 miRNAs	[85–88]
DNA damage/ PARP-1 signalling pathway	Activation of PARP-1 (a DNA repair enzyme) may lead to subsequent activation of transcription factors (NFAT, HIF-1α) that are implicated in PAH Inflammation induces DNA damage in PASMCs (levels of baseline and mutagen-induced DNA damage are intrinsically higher in PAH cells), which may lead to activation of PARP-1 in PAH	DNA damage/PARP-1 signalling pathway	[82, 89]
VEGFR signalling	VEGF plasma levels are elevated in patients with severe PAH, and expression of VEGF and VEGFR2 is robust in complex vascular lesions of PAH lungs Role of VEGF in mechanisms of PAH development is not clear	VEGFR signalling	[90]

HES5: hairy and enhancer of split 5; PARP-1: Poly [ADP-ribose] polymerase 1; VEGFR: vascular endothelial growth factor receptor; PASMCs: pulmonary artery smooth muscle cells; PH: pulmonary hypertension; STAT3: Signal transducer and activator of transcription 3; NFAT: nuclear factor of activated T-cells; HIF-1α: hypoxia-inducible factor-1α; PIM1: proto-oncogene serine/threonine-protein kinase; SMC: smooth muscle cell; SOD2: superoxide dismutase 2; HDACs: histone deacetylases; BRD4: bromodomain-containing protein 4; miRNAs: microRNAs; VEGF: vascular endothelial growth factor.

Loss-of-function mutations in *BMPR2* are present in up to 80% of HPAH and 11–40% of idiopathic PAH (IPAH) patients [22]. In the absence of a *BMPR2* mutation, reduced pulmonary vascular expression of BMPR-II has also been observed in PAH patients [23]. In a recent study by ROTHMAN *et al.* [24] the SMAD-specific E3 ubiquitin protein ligase 1 and miR-140-5p were identified as regulators of BMPR-II signalling and may represent a mechanism by which BMPR-II expression is reduced in PAH patients without *BMPR2* mutations.

Although the majority of patients with HPAH have heterozygous mutations in the *BMPR2* gene, the penetrance of *BMPR2* mutations is only 14–42%, indicating that other factors are involved in the pathogenesis of PAH [25]. Inflammation may be a trigger and this is supported by the observation that the presence of a heterozygous *BMPR2* mutation in mice did not lead to PH in unstressed conditions [26]. However, following introduction of an inflammatory stress, an increase in right ventricular systolic pressure was observed [26]. Another recent study identified a role for tumour necrosis factor (TNF)-α in the development of PH in the context of BMPR-II deficiency [27]. In this study, TNF-α-mediated suppression of BMPR-II led to pulmonary artery SMC proliferation. These alterations were confirmed in both an experimental PH animal model and in lung tissue from PAH patients. Furthermore, the introduction of TNF-α to SMCs from isolated HPAH pulmonary artery exacerbates the reduced expression of BMPR-II [27].

As *BMPR2* mutations are the most common genetic alteration associated with PAH, it is reasonable to hypothesise that augmenting BMP signalling may provide a therapeutic benefit to patients with PAH. BMPR-II agonists and drugs that non-specifically enhance BMP signalling have shown beneficial effects in experimental PH [24, 28–31]. In cultured ECs, BMP9 was shown to increase BMPR-II signalling, prevent apoptosis, decrease angiogenic behaviour and promote monolayer integrity [31]. Furthermore, administration of etanercept (a TNF-α inhibitor) reversed PH progression in a rat model, restoring BMP signalling and reducing right ventricular systolic pressure, right ventricular hypertrophy and muscularisation of small arterioles [27].

Platelet-derived growth factor and FGF signalling

Overexpression of platelet-derived growth factor (PDGF) and FGF has been implicated in the development of PAH [33]. PDGF induces the proliferation and migration of SMCs and fibroblasts [34]. Increased expression of PDGF-A, PDGF-B and their receptors (PDGFR-α and -β) has been reported in the pulmonary arteries of patients with PAH [35]. There are a number of factors that could lead to increased PDGF expression in PAH, including genetic predisposition and increased shear stress [33, 34]. This may then lead to an undesirable feedback loop, where upregulation of PDGF drives pulmonary vascular remodelling, leading to further shear stress and upregulation of PDGF and PDGFR [33].

Increased FGF-2 levels have been reported in experimental and human PH [36, 37]. FGF-2 is synthesised in a number of cell types, including ECs and fibroblasts, and exerts its influence through FGF receptors, which are expressed on the surface of different cell types, including vascular cells. Overproduction of FGF-2 by pulmonary ECs is thought to be involved in abnormal cross-talk between ECs and SMCs in PAH [36]. Furthermore, EC-derived FGF-2 is overproduced in IPAH and can contribute to the proliferation of cultured SMCs [36]. FGF-2 can also act in an autocrine manner to enhance proliferation and decrease apoptosis of ECs [38].

Given the extensive research on growth factors, there has been much interest in investigating growth factors as therapeutic targets in PAH. A long-term efficacy and safety phase III trial of the PDGFR tyrosine kinase inhibitor imatinib has been performed. However, despite improvements in exercise capacity and haemodynamics observed in patients who tolerated the drug, serious adverse events and premature treatment discontinuations were common [39]. The risks of most tyrosine kinase inhibitors that have been proposed as therapies for PAH (e.g. imatinib, sorafenib) currently outweigh the benefits, although the potential improvement in haemodynamic parameters may warrant further investigation with careful attention to identifying potential markers of a positive clinical response before or early in treatment [40].

Inflammation and immunity

There is a body of evidence to suggest that altered immune mechanisms play a significant role in the development of PAH via recruitment of inflammatory cells, response of the vascular cells to inflammatory stimuli, and autoimmune responses [46–48]. Inflammatory responses to infectious agents, including viruses and parasites, have also been implicated in the pathogenesis of PAH [49].

Pulmonary vascular lesions identified in patients with PAH, as well as in animal PH models, have been found to contain a number of immune cell types, including T- and B-lymphocytes, macrophages, dendritic cells and mast cells [50]. Lungs from IPAH patients have also been shown to contain perivascular tertiary lymphoid tissues, consisting of B- and T-cell areas with high endothelial venules and dendritic cells [51]. In IPAH patients, elevated circulating cytokine levels have been reported, some of which are involved in SMC proliferation [52].

Distal ECs respond to high pulsatile flow in arteries with an acute inflammatory response [16] and ECs and SMCs in the pulmonary vasculature can proliferate, migrate or develop an apoptosis-resistant phenotype in response to inflammatory stimuli [46]. Furthermore, pulmonary vascular remodelling can occur in response to inflammatory cytokines [53]. In an experimental model of PH, inflammation associated with CD4 immunodeficiency may be a predisposing factor for PH development [94]. In support of this, patients with certain PAH-associated conditions, e.g. systemic sclerosis, HIV infection and systemic lupus erythematosus, have previously been shown to exhibit abnormalities in T-regulatory cell number and function [54–56]. This may render these patients more susceptible to pulmonary vascular inflammation and remodelling after initial vascular injury [94]. However, the exact nature of inflammation as a cause or effect is still unknown in IPAH.

Although current PAH treatments possess immunomodulatory properties, there are no approved drugs specifically targeting inflammatory processes. However, a number are being evaluated in clinical trials [45, 57]. Increased inflammatory mediators are frequently observed in the various aetiologies of PAH, but only a small percentage of patients with established PAH (mostly those with active autoimmune diseases) respond to anti-inflammatory drugs [58].

Resting membrane potential

In 2013, mutations in the potassium channel subfamily K member 3 (KCNK3 or TASK-1) gene were reported in a family of patients with HPAH [59]. Subsequently, in a case series of 92 patients with HPAH (unrelated to the initial family) and 230 patients with IPAH, loss of function heterozygous mutations in the KCNK3 gene were identified in 3.2% of the HPAH and 1.3% of the IPAH patients [59]. A homozygous mutation in the KCNK3 gene has also been reported in a patient with a severe and early form of PAH who did not carry any other mutations known to be associated with PAH [60]. Potassium

channels can modulate vascular tone through regulation of the resting membrane potential in a number of cell types, including SMCs [61, 62], and may also play a role in the regulation of cell proliferation and migration [63].

The pathophysiological role of KCNK3 in PAH is unclear, but reduced expression and activation is thought to contribute to vasoconstriction and pulmonary artery remodelling *via* SMC and EC dysfunction [64]. This was suggested in a recent study by ANTIGNY *et al.* [64] which demonstrated that KCNK3 inhibition in an animal model is associated with increased proliferation of vascular cells, vasoconstriction and inflammation, all of which are known to contribute to PAH progression. Loss of KCNK3 activity is associated with key processes in PAH pathogenesis and it may therefore be a useful therapeutic target. In support of this, ANTIGNY *et al.* [64] reported that *in vivo* pharmacological activation of KCNK3 reduced right ventricular systolic pressure, right ventricular hypertrophy and pulmonary vascular wall thickness in a rat model of PH.

PAH is also associated with dysregulation of the potassium channel Kv1.5, which is encoded by the *KCNA5* gene. Expression of Kv1.5 is reduced in human and experimental PAH [65, 66] and *in vivo* gene transfer of Kv1.5 was shown to reduce PH in rats exposed to chronic hypoxia [67]. The mechanisms leading to its reduced expression in PAH are unclear at the current time.

Oestrogen signalling

It has long been reported that females are at higher risk of developing PAH compared with males [95]. Indeed, major registry studies have reported marked sex differences, with between 1.8 to 3.9-times as many females included as males [96–102]. For patients with *BMPR2* mutations, the risk of developing PAH is strongly influenced by sex: penetrance has been reported to be 14% for males and 42% for females in the Vanderbilt Pulmonary Hypertension Registry [25]. The reason for this is unclear, but many studies have implicated sex hormones as mechanistic factors in the development of PAH [95].

Recent interest in the relationship between sex hormones and PAH has been provoked by the observation that oestradiol (E2) metabolites may play a role in PAH pathogenesis. E2 can be converted to oestrone, which is then metabolised to 16α-hydroxyoestrone (16αOHE), a molecule known to have proliferative and inflammatory activities [95]. Alternatively, the CYP1A1 and CYP1B1 enzymes can convert E2 to metabolites such as 2-hydroxyoestradiol (2-OHE2) and 2-methoxyoestradiol (2-ME2), which are known to exert anti-proliferative and anti-inflammatory effects [95]. The differential effects of these metabolites on the development of PH have been demonstrated in a number of preclinical studies. Chronic exposure of *BMPR2* mutant mice to 16αOHE was associated with increased PH penetrance and severity compared with mutant mice not exposed to 16αOHE [68]. Conversely, studies have demonstrated that 2-OHE2 and 2-ME2 may exert protective effects in monocrotaline-induced PH in rats [69, 70]. Due to their potentially opposing actions, it has been hypothesised that an imbalance in the production of E2 metabolites may play a role in human PAH pathogenesis. This is supported by AUSTIN *et al.* [71] who demonstrated that PAH-affected *BMPR2* mutation carriers have a lower ratio of urinary 2-OHE2 to 16αOHE compared with unaffected *BMPR2* mutation carriers. This may be due to differences in CYP1B1 activity, as homozygosity for a CYP1B1 genetic polymorphism that has been implicated in several oestrogen-regulated cancers was more common in affected mutation carriers compared with unaffected mutation carriers [71]. These data suggest that if the balance of E2 metabolism shifts towards the production of the pro-proliferative 16αOHE metabolite, this can increase the risk of PAH in individuals who are already predisposed to the disease.

The exact mechanism by which 16αOHE can promote the pathogenesis of PAH remains to be elucidated. However, a recent study by CHEN *et al.* [72] has indicated a possible role for the miR-29 family. In this study, exposure to 16αOHE led to increased expression of miR-29 in *BMPR2* mutant mice. Furthermore, a beneficial effect of miR-29 antagonism was reported in terms of BMPR-II associated PH penetrance and severity in response to 16αOHE exposure in these mice. Elevated expression of miR-29 was also observed in lung tissue from HPAH patients, suggesting that this mechanism may also be relevant to the pathogenesis of human PAH. Of note, the miR-29 family has previously been linked to the regulation of glucose and lipid metabolism. This is of particular interest given the known association between PAH and the metabolic syndrome [73].

In addition to the role of its metabolites, E2 itself may directly protect against the development of PH. This hypothesis was explored in a study by LAHM *et al.* [74] which demonstrated that E2 attenuates the decline in haemodynamic and remodelling parameters observed in hypoxia-induced PH in rats. In this model, the benefits of E2 are mediated by the oestrogen receptor and conversion to E2 metabolites is not required [74]. However, a protective role for E2 in human PAH has yet to be demonstrated.

There is now accumulating evidence to support a role for E2 and its metabolites in the pathogenesis of PH. As such, this pathway may be another potential therapeutic target. Despite evidence to support the benefits of E2 in rat hypoxia-induced PH [74], the strong female predominance in human PAH makes E2 less appealing as a potential therapy as there are concerns that E2 may promote PAH in humans. Instead, targeting the pathways downstream of E2, *e.g.* the miR-29 family, may provide a means of treating PAH without the need to modulate sex hormones directly [72].

Iron homeostasis

The importance of iron status in PAH is illustrated by the fact that iron deficiency is prevalent in patients with IPAH and is associated with disease severity [76–78]. Furthermore, two studies in PH have demonstrated that the presence of anaemia is indicative of a poor prognosis [103, 104]. In individuals exposed to high altitude, PH can develop in response to hypoxia [105, 106], and two studies have demonstrated that iron may play a role in this process. In a study by SMITH *et al.* [79] iron supplementation reduced the increase in pulmonary artery pressure that occurs in response to an acute hypoxic challenge in healthy volunteers. In contrast, iron depletion exacerbated the response of the pulmonary vasculature to hypoxia [79]. A subsequent study by the same authors demonstrated that altitude-induced hypoxic PH could be attenuated by iron supplementation and exacerbated by iron depletion [80].

Patients can become iron deficient because of reduced intake, impaired uptake or increased loss of iron. In a study by RUITER *et al.* [77] oral iron therapy was ineffective at restoring normal ferritin levels in eight out of 18 IPAH patients, suggesting that, at least in some patients, a problem with absorption may be responsible for iron deficiency. In support of this, hepcidin, an inhibitor of dietary iron absorption, was shown to be elevated in a population of IPAH patients compared with healthy controls [76]. In the same study, a reduction in BMPR-II expression was associated with an increase in hepcidin expression *in vitro*. Given that reduced BMPR-II signalling is associated with PAH, this suggests a potential mechanism to explain the hepcidin elevation observed in PAH patients.

Although iron deficiency is prevalent in patients with PAH, the exact relationship between iron and PAH pathogenesis is unknown. A study by COTRONEO *et al.* [81] has demonstrated for the first time that pulmonary vascular remodelling can occur as a result of chronic iron deficiency in rats. Further experiments indicated that this may result from metabolic changes induced by iron deficiency, including a shift towards aerobic glycolysis [81]. In this animal model, pulmonary vascular remodelling was reversed by iron supplementation, supporting its use as a potential treatment option in PH patients [81].

Conclusions

In PAH, three well-characterised pathogenic pathways are currently targeted by approved therapies. However, there are many other complex and often interacting pathways involved in the development and progression of the disease. Work is ongoing to elucidate these pathways and increase our understanding of the pathogenesis of PAH. As more data become available on these novel targets, it may become feasible to prioritise which targets should enter clinical development. In recent years, the use of combination therapy to target multiple pathogenic pathways simultaneously has improved outcomes for PAH patients. The development of additional therapies with novel modes of action might further increase our ability to impede pathological changes within the pulmonary vasculature and thus delay the progression of PAH.

Acknowledgements

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