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The role of endothelial cells in pulmonary hypertension: old concepts and new science

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Endothelial dysfunction plays a key role in the initiation and progression of pulmonary hypertension (PH), a fatal and currently incurable disease associated with increased pulmonary vascular resistance and leading to right heart failure. Recent data from genomic, transcriptomic and metabolomic studies and emergence of novel organ-on-chip technologies have provided new insights into the role of endothelium in the pathogenesis of this disease. In this review, we characterise key features of endothelial dysfunction in PH, highlighting the diversity of endothelial cell types and differences in their responses to vascular insults. We also present the current understanding of the effects of genetic mutations on endothelial function and comment on new ways of disease modelling using organ-on-chip technologies.

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Introduction

Pulmonary hypertension (PH) is a vascular lung disorder clinically characterised by increased mean pulmonary arterial pressure > 20 mm Hg and pulmonary vascular resistance ≥ 3 Wood units, which leads to right ventricular hypertrophy and often results in right heart failure. PH is an umbrella term for a range of disorders classified into 5 groups, according to the disease aetiology: Group I: pulmonary arterial hypertension (PAH); Group II: PH due to left heart disease; Group III: PH due to lung diseases and/or hypoxia; Group IV: PH due to pulmonary artery obstructions; Group V: PH with unclear and/or multifactorial mechanisms [1]. Despite extensive research, the existing therapies bring only a symptomatic relief and PH remains incurable.

Regardless of differences in the disease aetiology, all functional classes of PH are characterised by progressive narrowing of small arteries that carry oxygen-poor blood to the lung. This increases mechanical load on the right ventricle, leading to right ventricular hypertrophy. Thickness of arterial intima, normally consisting of a single layer of endothelial cells (ECs), increases 2–3 fold in PAH [2,3]. A hallmark of advanced human PAH is the formation of occlusive plexiform lesions, often located at vascular branching points [4]. They comprise mainly proliferating ECs forming disorganised angiogenic vascular channels, but also smooth muscle cells (SMCs), fibroblasts, inflammatory cells and microthrombi [5,6]. Their origin and contribution to PAH pathogenesis are not clear, but one theory is that they may represent anastomosing structures between bronchial microvessels and pulmonary vasculature [7].

PH, such as many other cardiovascular disorders, is considered a multifactorial disease and the current view is that it is initiated by a combination of two or more 'hits' such as genetic mutations or epigenetic alterations such as changes in DNA methylation, histone modifications, microRNAs, long noncoding RNAs and histone methylation, as well as hypoxia, mechanical stress, infection or drugs and toxins [8–10].

Endothelial dysfunction in pulmonary hypertension

PH is thought to be initiated by endothelial injury, leading to EC senescence and apoptosis followed by exaggerated repair, which results in the formation of proliferative vascular occlusions [11,12]. This view is predominantly based on observations from animal models, where early endothelial apoptosis and pulmonary vessel rarefaction were followed by endothelial proliferation [13].

In general, PH endothelium presents an altered paracrine signalling manifested by increased release of vasoconstrictors, such as endothelin-1 and thromboxane and reduced release of vasorelaxants, such as nitric oxide and prostacyclin. In addition, ECs release a range of growth factors, cytokines and chemokines facilitating vascular remodelling and fuelling inflammatory and prothrombotic responses. Under these conditions, endothelial barrier function is compromised, allowing the leakage of blood components to the underlying vascular tissues, stimulating medial cell proliferation, migration,

thrombosis and inflammatory cell activation. ECs may also contribute to arterial remodelling via endothelial-to- mesenchymal transition, a process by which they acquire proliferative, mesenchymal phenotypes [14]. In addition to this, metabolic shift from oxidative phosphorylation to glycolysis helps provide the energy and essential metabolites important to sustain the heightened proliferative potential of pulmonary vascular cells [15]. Endothelial dysfunction in PH is likely to develop gradually and involves altered interactions with other cell types residing in vasculature and the surrounding tissues. For example, loss of endothelial–pericyte (PC) interactions resulting from PC metabolic reprogramming, has been linked to progressive small-vessel loss in PAH [16].

Diversity of endothelial cell phenotypes in pulmonary hypertension

Advances in the 'omics' technologies in the past few years uncovered new facts regarding endothelial function in PH, often challenging the more established views. A high-resolution analysis of single-cell RNA sequencing (scRNA-seq) data of lung ECs from Sugen/hypoxia mouse model of PAH revealed that disease-related changes occur within diverse EC populations, belonging to arterial, venous, lymphatic vessels and two discrete populations of capillary ECs [17]. All EC populations showed upregulation of the major histocompatibility complex class-II pathway, supporting the view of contributory role of inflammation in PAH, but only a subset of capillary ECs showed upregulation of genes involved in cell death, cell motility or angiogenesis. Contrary to the existing views, this study found little evidence of endothelial-to-mesenchymal transition (EndoMT) or EC proliferation. *In silico* cell ordering approach identified zonation-dependent changes across the arteriovenous axis in mouse PAH and showed upregulation of angiogenesis regulators, serine/threonine-protein kinase Sgk1 at the junction between the macro- and microvasculature and upregulation of two secreted proteins acidic and rich in cysteine family proteins in capillary cells [17]. The significance of these findings in the context of human PAH will require further studies, but it is becoming apparent that the term 'endothelial dysfunction' means different things, depending on the cell type, location and the stage of the disease.

The analysis of human lung samples from idiopathic PAH using droplet-based scRNA-seq reinforced the view of endothelial contribution to PAH and identified novel differentially expressed genes. At this stage, no changes in endothelial proliferation markers were noted. Interestingly, the study highlighted the role of PCs which, together with endothelial and SMCs, had the most differentially expressed gene profile compared with other cell types [18].

Endothelial dysfunction — lessons from genome sequencing

Genetic deficiency increases susceptibility to PAH, but its impact on endothelial function is not well understood. In addition to the most common heterozygous germline mutations in the *bone morphogenetic protein receptor 2* (*BMPR2*) gene, other gene mutations have been described in hereditary PAH, with aquaporin 1 *(AQP1)*, polyamine-transporting ATPase 13A3 *(ATP13A3)*, sex-determining region Y protein (SRY-box) transcription factor 17 *(SOX17)*, ATP-binding cassette subfamily C member 8 *(ABCC8)*, gamma-glutamyl carboxylase *(GGCX)*, kallikrein 1 *(KLK1)* and kinase insert domain receptor (*KDR)* most recently identified by whole-exome or whole-genome sequencing [9]. Predicted function of the gene products in ECs is summarised in Table 1. While this analysis may provide an indication of the key pathways associated with the disease, delineation of specific mechanisms regulating endothelial function at various stages of the disease in different types of pulmonary vascular cells will require further studies.

Circulating endothelial progenitor cells

Eendothelial progenitor cells (EPCs), first isolated from peripheral blood samples in 1997 by Asahara et al., have provided a valuable tool for modelling of cardiovascular diseases [66]. These CD34+ or KDR+ mononuclear cells were shown to differentiate into ECs in cell culture. Ambiguity associated with terminology and function of EPCs, revealed in subsequent studies, has been described in recent reviews [67,68]. It is now accepted that cultured peripheral blood mononuclear cells can differentiate into 2 lineages — the late-outgrowth endothelial colony- forming cells (ECFCs) and the myeloid angiogenic cells, each type expressing a set of specific differentiation markers [68]. ECFCs are characterised by a potent reparative and angiogenic potential and are thought to be mobilised in response to endothelial injury.

ECFCs from PAH patients were shown to replicate key features of endothelial dysfunction in the disease, including the heightened proliferative potential and abnormal angiogenesis, and therefore have been used in studies on disease mechanisms and to validate findings obtained with animal models [69–71]. Therapeutic effects of EPCs demonstrated in preclinical models of PAH were associated with improvement of immune responses [72], or accelerated endothelial healing of the damaged pulmonary arterioles [73].

Organ-on-a-chip modelling of endothelial dysfunction in pulmonary hypertension

The existing preclinical models of PH cannot accurately recapitulate pathology of human disease and this

Table 1

Effect of PAH-associated genetic mutations on endothelial function.

Gene symbol	Function	Name	Predicted function of the gene products in ECs	Ref.
ABCC8 (SUR1)	Regulatory subunit of the ATP-sensitive potassium channel (KATP) channel	ATP-binding cassette subfamily C member 8	Regulation of endothelial barrier function, cell proliferation and inflammatory responses	[19–22]
ACVRL1 (ALK1)	Encodes transmembrane T1 receptor	Activin-A receptor- like type 1	Regulation of suppressor of mothers against decapentaplegic (SMAD1/5/8) signalling, endothelial migration and proliferation, angiogenesis	[23–25]
AQP1	Membrane channel allowing rapid water movement	Aquaporin 1	Regulation of cell migration and proliferation	[26,27]
ATP13A3	Encodes P-type enzyme that hydrolises adenosine triphosphate (ATPase), transports cations across membranes	Polyamine-transporting ATPase 13A3	Regulation of cell proliferation and apoptosis	[27,28]
BMP10	BMP-9 and BMP10 circulate in plasma at physiologically active levels and bind selectively to the endothelial receptor complex activin-A receptor- like type 1 (ALK1)/BMPR2	BMP 10	Regulation of Alk1 and Smad1/5/9 signalling, angiogenesis, endothelial homoeostasis	[29,30]
BMPR1B (ALK6)	Transmembrane serine/threonine kinase, bone morphogenetic protein (BMP) receptor	Bone morphogenetic protein receptor, type 1B	Regulation of EC proliferation and angiogenesis	[31,32]
BMPR2	Encodes transmembrane type-II receptor	Bone morphogenetic protein receptor, type II	Regulation of EC proliferation, migration, barrier function, angiogenesis and apoptosis	[24,33,34]
CAV1	Cav1 regulates endothelial response to shear and endothelial nitric oxide synthase (eNOS) activity	Caveolin 1	Regulation of endothelial permeability, TGFβ1-mediated activation of SMAD2/3, VEGF signalling, EC proliferation	[24,35–37]
EDN1 (ET-1)	Endothelium-derived vasoactive peptide	Endothelin-1	Regulation of EC migration, angiogenesis, proliferation, EC survival, vascular fibrosis, thrombosis, vasoconstriction, inflammation	[38–40]
ENG	Type-I transmembrane glycoprotein, ligand of the transforming growth factor beta (TGF-β)	Endoglin	Regulation of EC proliferation, angiogenesis, inflammation, cell differentiation, endothelial regulation of vascular SMC proliferation	[23,41]
GDF2	Encodes BMP-9, a ligand for ALK1 and BMPRII in pulmonary EC	Growth differentiation factor 2	Regulation of endothelial homoeostasis, SMAD signalling, endothelial barrier function, angiogenesis, inflammatory responses	[29,42, 43]
GGCX	Enzyme essential for biosynthesis of vitamin-K-dependent clotting factors	Gamma-glutamyl carboxylase	Regulation of EC proliferation and inflammatory responses	[44]
KCNK3	pH-sensitive potassium channel, controls the resting membrane potential in cells	Potassium two- pore domain channel subfamily K member 3	Regulation of EC proliferation, apoptosis and inflammatory responses	[22,45]
KDR	Encodes vascular endothelial growth factor receptor 2 (VEGFR2)	Kinase insert domain receptor	Regulation of VEGF signalling, endothelial proliferation, apoptosis and angiogenesis	[46,47]
KLK1	Serine protease	Kallikrein 1	Regulation of NO/cGMP signalling, coagulation, cell adhesion and inflammation	[44,48,49]
NOTCH3	Receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1	Neurogenic locus notch homologue protein 3	Regulation of endothelial barrier function, EC apoptosis and angiogenesis	[50]
SIRT3	Mitochondria-located deacetylase Sirtuin 3, maintains mitochondrial homoeostasis	Sirtuin 3	Regulation of mitochondrial function, ROS generation, glycolytic reprogramming, apoptosis, proliferation and angiogenesis	[51,52]
(SMAD1)	Regulates BMP signalling, complexes with Smad 5 to activate Smad4 and induce its translocation to the nucleus. Canonical SMAD pathway regulates EC proliferation by interacting with the neurogenic locus notch homologue (NOTCH) pathway to upregulate angiogenic genes	Smad family member 1	Regulation of endothelial proliferation, migration and angiogenesis	[53]

Table 1 (*continued*)

Gene symbol	Function	Name	Predicted function of the gene products in ECs	Ref.
SMAD4	Core component of the canonical TGF-β signalling pathway; mediates Smad1/5/9 effects on EC proliferation, migration and angiogenesis, regulates mechanotransduction	Smad family member 4, also called Mothers against decapentaplegic homologue 4	Regulation of TGF-β signalling, angiogenesis, EC responses to flow	[54,55]
SOX17	Transcription factor, regulator of endothelial lineage	SRY-Box transcription factor 17	Regulation of EC proliferation, inflammatory activation and arterial identity	[56]
TBX4	Transcription factor, regulates developmental processes	T-box 4	Regulation of endothelial maturation, Smad1,5 signalling and vessel formation	[57–60]
THBS1	Extracellular matrix glycoprotein	Thrombospondin 1	Regulation of endothelial proliferation, angiogenesis, apoptosis, TGF-β1 signalling	[61,62]
TOPBP1	Maintenance of the genomic integrity by activating ataxia telangiectasia-mutated gene (ATM) and ataxia telangiectasia and Rad3-related (ATR)	Topoisomerase (DNA) II-binding protein 1	Regulation of DNA repair and angiogenesis	[63]
TRPC6	Ca^{2+}-permeable nonselective cation channel	Transient receptor potential cation channel, subfamily C, member 6	Regulation of endothelial barrier function, leucocyte transmigration and vascular endothelial growth factor (VEGF)-induced angiogenesis	[64,65]

ETA: endothelin receptor A *(EDNRA) (ETA), which is not expressed by ECs, is not included.*

significantly limits the drug discovery process. New technologies, called Organs-on-chips, which reproduce key structural and functional features of human organs, have emerged in the last decade with huge potential to revolutionise in vitro disease modelling [74].

Recent study by Ainscough et al. described a new inducible biomimetic organ-on-a-chip model of PAH utilising layered human pulmonary artery endothelial and SMCs cocultured under flow and exposed to a 'double hit' of hypoxia combined with *BMPR2* knockdown [75]. This model identified key transcriptomic and functional changes in human endothelium required for the induction of medial smooth muscle proliferation, including BMPR2-mediated downregulation of arterial identity factor SOX17. Consistent with the findings from human PAH, pulmonary artery ECs showed a loss of arterial identity, reduced eNOS signalling, increased susceptibility to damage and metabolic shift towards glycolysis. Interestingly, blood-derived ECFCs from patients with *BMPR2* mutations showed an opposite reaction to stress, characterised by upregulation of genes regulating nitric oxide (NO) bioavailability and endothelial repair [75], which may reflect their role in vascular regeneration PAH [76].

A different organ-on-a-chip model of PAH utilised intimal, medial and adventitial cells from PAH pulmonary arteries grown in straight channels arranged side-by-side [77]. This design can facilitate observation of cell behaviour and analysis of cell secretome, but is not suitable for studies on endothelial barrier function and is limited by poor accessibility of patient lung material.

Conclusions and future directions

Loss of endothelial homoeostasis is a key contributory factor in the pathogenesis of PH. Novel genomic, transcriptomic and metabolomic analyses uncovered high complexity in EC responses to the disease-related stimuli, depending on the type of host or preclinical model, EC type and the stage of the disease. Efforts should be made towards the integrative analysis encompassing data from different methodologies and disease models. Organ-on-a-chip disease models, which facilitate observations of patient-derived ECs cultured together with other cell types under physiologically relevant conditions, can provide important mechanistic insights into the role of endothelium in PH and help explain discrepancies between preclinical models and human condition.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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