Arteriosclerosis, Thrombosis, and Vascular Biology

ATVB IN FOCUS:
Pulmonary Vascular Biology

Series Editor: Akiko Mammoto

Endothelial Heterogeneity in Pulmonary Hypertension

Hanqiu Zhao, Murali M. Chakinala, Michael B. Fallon, Susan M. Lin, Janet S. Lee, Zhiyu Dai

ABSTRACT: The lung endothelium is essential for maintaining normal lung structure and plays a key role in gas exchange, barrier function, angiogenesis, vascular tone, and inflammation regulation. The advent of single-cell RNA sequencing has revealed the unique heterogeneity of pulmonary endothelial cells (ECs) in their function, morphology, and localization. Pulmonary hypertension (PH) is a progressive vascular disorder marked by elevated pulmonary arterial pressure and vascular remodeling. Central to its pathogenesis is EC dysfunction, and emerging evidence highlights EC heterogeneity in driving the complexity of PH. The distinct lung endothelial subpopulations exhibit diverse molecular signatures and functional responses under PH. A complete picture of how these different subpopulations contribute to vascular remodeling of PH is critical to identify novel therapeutic opportunities. This brief review summarizes recent insights into EC dysfunction in PH, focusing on the role of specialized EC subsets and novel therapeutic strategies targeting EC dysfunction. We highlight the integration of cutting-edge technologies in understanding how endothelial heterogeneity shapes the trajectory of PH and opens new avenues for future therapeutic innovations.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: capillaries ■ endothelial cells ■ hypertension, pulmonary ■ pulmonary arterial hypertension ■ vascular remodeling

Pulmonary hypertension (PH) is characterized by the mean pulmonary pressure >20 mm Hg, as measured by right heart catheterization when at rest. It is estimated that at least 1% of the world's population is affected by PH.[1] Pulmonary vascular remodeling is a common characteristic of all subtypes of PH. It encompasses structural and functional alterations in distal pulmonary arteries, capillaries, and small-to-medium–sized veins. These changes result in a persistent elevation of pulmonary vascular resistance, ultimately leading to an increase in pulmonary arterial pressure.[2]

Pulmonary endothelial cells (ECs) are essential for preserving normal lung structure and function. They play a pivotal role in gas exchange, barrier function, angiogenesis, vascular tone regulation, hemostasis, and the modulation of inflammation response.[3,4] The advent of single-cell RNA sequencing (scRNA-seq) has unveiled the unique heterogeneity of pulmonary ECs. Different EC subpopulations exhibit distinct features in localization, morphology, and transcriptomics profiles, contributing differently to the development and injury response.

The discovery of nitric oxide as the endothelium-derived relaxing factor initiated extensive investigations and contributed to the definition of EC dysfunction, initially described as impaired vascular tone resulting from an imbalance between relaxing and contracting factors.[5] Our understanding of EC dysfunction has expanded, now recognizing its involvement in vascular remodeling in PH through multiple mechanisms, including hyperproliferation, apoptosis resistance, altered metabolism, epigenetic regulation, and endothelial-mesenchymal transition (EndoMT).[6,7] In pulmonary arterial hypertension (PAH), single-cell transcriptomic profiling studies in mice and humans have demonstrated that EC subpopulations are skewed in disease states and possibly contribute to EC dysfunction.[8–10] However, much remains to be elucidated regarding how EC diversity shapes PH pathogenesis, particularly with the rise in cutting-edge technologies such as spatial omics. This review will summarize how EC heterogeneity contributes to PH, highlight emerging therapeutic strategies targeting dysfunctional ECs, and discuss the latest omics and other technical advances that enhance our understanding of PH.

Please see www.ahajournals.org/atvb/atvb-focus for all articles published in this series.

Correspondence to: Zhiyu Dai, PhD, Division of Pulmonary and Critical Care Medicine, Department of Medicine, Washington University School of Medicine in St. Louis, 4960 Children's Pl, St. Louis, MO 63110. Email z.dai@wustl.edu
For Sources of Funding and Disclosures, see page 12.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

aCap	aerocyte or Cap2
ALK	anaplastic lymphoma kinase
AT1	alveolar type I epithelial cell
EC	endothelial cell
ECM	extracellular matrix
EndoMT	endothelial-mesenchymal transition
gCap	general capillary endothelial cell or Cap1
HOP	homeodomain-only protein
IPF	idiopathic pulmonary fibrosis
LEC	lymphatic endothelial cell
MVEC	microvascular endothelial cell
PAH	pulmonary arterial hypertension
PDGF	platelet-derived growth factor
PDGFR	platelet-derived growth factor receptor
PECAM-1	platelet endothelial cell adhesion molecule-1
PH	pulmonary hypertension
PV	pulmonary vein
PVEC	pulmonary venous endothelial cell
scRNA-seq	single-cell RNA sequencing
SuHx	Sugen 5416/hypoxia
SVEC	systemic venous endothelial cell
TGF-βR2	transforming growth factor-β receptor 2
TMEM100	transmembrane protein 100
VEC	venous endothelial cell

Highlights

- Lung endothelial cells (ECs) are categorized into arterial ECs, capillary ECs, including aerocytes and general capillary ECs, venous ECs, including pulmonary venous ECs and systemic venous ECs, and lymphatic ECs.
- EC subtypes exhibit differential responses to environmental triggers, demonstrate plasticity during injury, and undergo phenotypic shifts that may contribute to the development and progression of pulmonary hypertension.
- EC-targeted nanomedicine and advent technologies such as spatial transcriptomics and organoid systems might be used to study pulmonary hypertension pathogenesis and treat pulmonary hypertension.

EC HETEROGENEITY IN THE PULMONARY VASCULATURE

The lung is a highly vascularized organ. ECs comprise ≈30% of cells in the healthy adult lung[11] and form the monolayer of the vascular and lymphatic tube intima. In addition to maintaining barrier homeostasis, ECs also contribute to gas and nutrient exchange, immune regulation, angiocrine signaling, and the regulation of vascular tone.[3]

A single-cell atlas has revealed the heterogeneity of lung ECs.[12–14] Currently, the lung ECs are categorized into 4 major subtypes: arterial ECs (AECs), capillary ECs, venous ECs (VECs), and lymphatic ECs (LECs).

In the healthy adult lungs, AECs are exposed to high shear stress and play a crucial role in regulating vascular tone and sensing mechanical stress. In humans, AECs highly express genes encoding gap and tight junction proteins, including *GJA4*, *GJA5*, and *CLDN10* (claudin), and actively release signaling molecules such as CXCL12 (stromal cell-derived factor 12), VEGFA (vascular endothelial growth factor A), and Ephrin-B2. They also express components of notch signaling (*DLL4*, *HEY1*, and *HES4*) and WNT pathway regulators such as *DKK2*[12] to preserve arterial identity (Table 1).

Capillary ECs constitute the majority of the cell population of the healthy lung. They play a crucial role in the blood-air barrier, possessing the unique ability to maintain barrier integrity and facilitate efficient gas exchange. Recent mouse studies initially provided insights into 2 intermingled subpopulations that comprise the capillary endothelium: general capillary EC (gCap, also named Cap1) and aerocyte (aCap, also named Cap2), which are distinct from each other both in function and morphology.[12,20,24] gCaps are smaller in size and less branched than aCaps. Studies in mice have shown that gCap possesses proliferative capacity, serving as the capillary EC stem cell reservoir, which contributes to EC replenishment during development and lung vascular injuries. gCaps are characterized by *Aplnr* (apeline receptor) and *Gpihbp1*, localizing to the thick region of alveolar walls, which is separated from the epithelium by stromal cells and connective tissue. In this niche, gCaps closely contact pericytes and fibroblasts,[20] presumably contributing to vascular homeostasis through Edn1 (endothelin 1) signaling from gCaps to Ednra (endothelin receptor type A) expressed in pericytes and fibroblasts.[20] gCaps highly express genes related to lipid metabolism (*Gpihbp1*), MHC II components (*Cd74*, *H2-aa*, and *H2-ab1*), and vasoconstrictor *Edn1* and *Nos3* (Table 1), suggesting its potential roles in metabolism, antigen processing, and vasomotor regulation in addition to injury repair.[20] aCaps are a unique lung-specific EC subtype characterized by their large size (often >100 μm) and porous Swiss cheese appearance.[20] They localize to the thin regions of the alveolar walls, where they are closely opposed to alveolar type I epithelial cell (AT1) across a shared basement membrane and are structurally specialized to facilitate efficient gas exchange.[20,24] aCap cells are defined by the high expression of carbon dioxide transport gene *Car4*, endothelin receptor *Ednrb*, *Hpgd*, and calcium binding protein *S100a4* and also express genes associated with leukocyte trafficking, such as *Icam1*, *Chst1*, and *Chst2*. The expression of Kdr and Ednrb in aCaps

Table 1. Marker Genes for Pulmonary EC Subtypes

Endothelial classification	Endothelial subtypes	Subdivision	Marker genes in human	References	Marker genes in mouse	References
Pan EC			*CDH5*, *CLDN5*, *PECAM1*, *ERG*, *TIE1*, *CAV1*, and *CAVIN2*	12,15,16	*Cdh5*, *Cldn5*, *Pecam1*, *Erg*, *Tie1*, *Tek*, *Cav1*, and *Cavin2*	12–14
			TMEM100	13,14,17	*Tmem100*	13,14,17
	AEC		*DKK2*, *GJA5*, *IGFBP3*, and *HEY1*	12,18,19	*Cxcl12*, *Gja4*, *Gja5*, *Sox17*, *Dkk2*, *Ltbp4*, *Efbn2*, and *Bmx*	12
			CXCL12, *GJA4*, *SOX17*, *LTBP4*, *EFBN2*, and *BMX*	12		
	Capillary EC		*CA4*	12,20	*Rgcc*, *Sparc*, and *Sgk1*	13
			RGCC	21		
			PRX	22		
			HLA-II	23		
		gCap (Cap1)	*VWF*^{pos}/*EMCN*^{low}/*EDN1*, *APLNR*, *GPIHBP1*, *FCN3*, and *EDN1*	12,20	*Gpihbp1*, *Plvap*, *Cd93*, *Ptprb*, *Aplnr*, and *Kit*	12,20
			IL7R and *SLC6A4*	12,19,20		
		aCap (Cap2)	*VWF*^{neg}/*EMCN*^{high}, *EDNRB*, *HPGD*, *APLN*, *KDR*, *TBX2*, *SOSTDC1*, and *S100A4*	12,20	*Car4*, *Ednrb*, *Apln*, *Hpgd*, *Kdr*, and *Tbx2*	12,20,24
			ACKR1	12,18,19		
	VEC		*NR2F2*, *VWF*, *IGFBP7*, *VCAM1*, *SELP*, *SELE*, *ADAMTS9*, and *HDAC9*	12		
		PVEC	*CPE*	12,18,19		
			COL15A1^{neg}, *DKK3*, *C3*, *CLU*, *PTGS1*, *PTGIS*, and *EPHB4*	12	*Ackr1*, *Nr2f2*, *Vwf*, *Slc6a2*, *Ephb4*, and *Hdac9*	12
		SVEC	*COL15A1*^{pos}, *PLVAP*, and *SPRY1*	12,18,21,25		
			EBF1, *MEOX2*, *TSHZ2*, *VWA1*, *SPARCL1*, *NRP2*, *HSPG2*, and *MAGI1*	12		
			ZNF385D	12,18,21,25		
LEC			*CCL21* and *PDPN*	12,19	*Prox1*	12,13,26
			PROX1, *LYVE1*, *FLT4*, and *SEMA3D*	12,18	*Lyve1*	12,13,27
					Pdpn	12,13
			MMRN1, *SEMA3A*, *TBX1*, and *KLHL4*	12	*Mmrn1*, *Sema3d*, *Tbx1*, *Klhl4*, *Ccl21b*, *Thy1*, and *Tbx1*	12

aCap indicates aerocyte; AEC, arterial endothelial cell; EC, endothelial cell; gCap, general capillary endothelial cell; LEC, lymphatic endothelial cell; PVEC, pulmonary venous endothelial cell; SVEC, systemic venous endothelial cell; and VEC, venous endothelial cell.

suggests dynamic interactions with AT1s, AECs, and gCaps, all of which are capable of releasing the corresponding ligands VEGFA and endothelin-1,[12,24,28,29] while aCap-derived ligands Apln (apelin) and Kit (KIT proto-oncogene receptor tyrosine kinase) act on the receptors Aplnr and Kit in gCaps, indicating a bidirectional regulatory crosstalk between these 2 capillary EC subtypes.[12]

VECs are characterized by the expression of *ACKR1*, which is involved in immune cell trafficking, as well as transcriptional factor *NR2F2* (nuclear receptor subfamily 2, group F, member 2), playing a key role in suppressing arterial identity (Table 1). Recent studies in the healthy human adult lungs, as well as patients with idiopathic pulmonary fibrosis (IPF), revealed that VECs are further divided into 2 populations: *COL15A1*^{neg} pulmonary venous EC (PVEC) and *COL15A1*^{pos} systemic venous EC (SVEC).[12,25] PVECs, originating from pulmonary veins (PVs) where oxygen-rich blood is transported to the left atrium of the heart, are marked by carboxypeptidase *CPE* and WNT modulator *DKK3*. PVECs also express genes related to prostaglandin synthesis (*PTGS* and *PTGIS*) and genes involved in the complement and coagulation cascade (*C7*, *PLAT*, *PROCR*, and *CLU*).[12,13] In addition to highly expressing *COL15A1*, which encodes the ECM (extracellular matrix) structural constituent-alpha chain of type XV collagen, SVECs possess a unique transcriptomic profile that includes *VWA1* and *PLVAP*, genes potentially related to ECM organization and regulation of vascular permeability, respectively.[12,21,30–32] Immunostaining showed that SVECs are located in the bronchial vascular plexus and visceral pleura of the healthy human lung.[12,25] The distinct gene expression profiles of PVECs and SVECs not only highlight their molecular heterogeneity but also suggest that PVECs are functionally more specialized to support lung-specific vascular roles. Several studies have shown that VECs serve as a primary source of de novo angiogenesis in response

to *VEGFA* during development and contribute to the repair of the lung capillary bed following injury.[33,34] These findings have drawn increasing attention to the progenitor potential of VECs.

LECs originate from venous-derived lymph sacs during development[35] and form the endothelial lining of lymphatic vessels, which transport interstitial fluid to the lymph nodes and collective veins.[36] LECs also control trafficking and responses of immune cells.[35,37,38] LECs are distinguished by canonical lymphatic markers: *PROX1*, *FLT4*, *PDPN*, and *LYVE1*[35] (Table 1). LECs also express *SEMA3A* (semaphorin 3A) and *SEMA3D*, genes encoding secreted proteins that play crucial roles in directing the maturation and patterning of lymphatic vessels.[12,39,40] In addition, bioinformatic analysis indicates that LECs have extensive communication with other cell types. Expression of *CCL21* in LECs suggests regulation of T cells and dendritic cells recruitment. Cell communication analyses also show LECs interact with pericytes and smooth muscle cells via the PDGF (platelet-derived growth factor)-A/PDGFR (platelet-derived growth factor receptor)-β signaling axis. In addition, LEC-secreted SEMA3A contributes to intercellular communication by binding to its receptors, NPR1, PLXNA1/2 (plexin A1/2), and PLXNA4 (plexin A4), which are expressed in various cell types, including fibroblasts, ECs, AT1/alveolar type II epithelial cells, mesothelial cells, and dendritic cells.[12,13]

Although EC subtypes and marker genes are largely conserved between mice and humans, several significant distinctions in lung ECs warrant attention. In the human lung, *IL7R* is highly expressed in gCaps, serving as one of the key marker genes for gCaps.[20] Conversely, in mice, *Il7r* expression is predominantly restricted to immune cells, specifically in T cells, but not gCaps.[41,42] Furthermore, *PLVAP* has been identified as an SVEC marker in human lungs[12] and is expressed in ECs of other organs, including liver, brain, and heart. The physiological function of PLVAP in SVECs and gCaps remains unclear. In cancer research, PLVAP has been implicated in angiogenesis across various tumor types and is widely recognized as a marker of tumor-associated ECs. Studies have demonstrated an expansion of *VWA1*^{pos}/*PLVAP*^{pos} ECs in IPF[31] and *ACKR1*^{pos}/*PLVAP*^{pos} ECs in the fibrotic niche of liver cirrhosis.[43] These findings suggest a conserved and significant role for SVECs in fibrotic diseases across organs. In contrast, the systemic circulation supplying the main bronchus and large vessels in mice does not penetrate into the lung mesenchyme[44]; thus, SVECs are rarely detected in healthy adult mouse lungs. In addition, given that *VWF* is consistently expressed at higher levels in VECs across EC subtypes, it is commonly used as a marker for VEC in scRNA-seq annotations. In humans, the absence of *VWF* in aCaps makes it serve as a good marker to differentiate aCaps from gCaps, whereas, in mice, *Vwf* is absent in both aCaps and gCaps. On the other hand, *Plvap* expression in mouse ECs is predominantly observed in gCaps and in ECs of large vessels including VECs and AECs. Therefore, *Plvap* is utilized to distinguish gCap from aCap populations in capillaries in the mouse.[20] In contrast, *Car4* is predominantly expressed in aCaps in adult mouse lungs, whereas *CA4* is generally regarded as a pan-capillary EC marker in humans.[12,20] These interspecies differences in gCap marker expression suggest that human gCaps may possess broader functional roles compared with their murine counterparts. The heterogeneity of EC across species, particularly gCaps with regenerative potential, necessitates increased attention when annotating or integrating mouse and human single-cell data sets. This underscores the importance of selecting appropriate model organisms (Table 2) and cell markers for lung endothelium research.

EC HETEROGENEITY IN PH

PH is a progressive disease characterized by a high mortality rate. It is classified into 5 subgroups, including group 1 PAH, group 2 PH associated with left heart disease, group 3 PH associated with lung diseases, group 4 PH associated with pulmonary arterial obstructions, and group 5 PH with unclear and multifactorial mechanisms.[63] Pulmonary vascular remodeling is a general pathological process found in the 5 groups of PH, involving structural and functional alterations of distal pulmonary arteries, capillaries, and postcapillary veins to various degrees. Emerging evidence suggests that pulmonary EC subpopulations that have undergone shear stress, either in vitro or during the development of PH, exhibit altered transcriptomic and proteomic profiles.[8,10,64] Furthermore, each subgroup of PH is associated with characteristic vascular lesions: plexiform lesions are the hallmark of PAH; muscularization of PVs and venules are central to group 2 PH; patients with chronic obstructive pulmonary disease and IPF with severe PH are often observed with capillary rarefaction and increased microvascular remodeling; and lungs from patients with chronic thromboembolic PH often exhibit recanalized thrombotic lesions in pulmonary arteries. Therefore, understanding EC heterogeneity is crucial for advancing research across PH subgroups. EC dysregulation regarding proliferation, migration, oxidative stress, metabolism, and epigenetics in PH has been comprehensively reviewed elsewhere.[2,6,65] In this review, we highlight recent findings demonstrating that EC subtypes exhibit differential responses to environmental triggers, demonstrate plasticity during injury, and undergo phenotypic shifts that may contribute to the development and progression of PH (Figure).

AEC

AECs are exposed to high shear stress and are highly sensitive to mechanical cues, which are markedly altered in the pulmonary circulation during PH. Shinohara et al[66]

Table 2. Available Mouse Cre Lines for Pulmonary EC Subtypes

Endothelial classification	Endothelial subtypes	Subdivision	Available mouse Cre lines	References
Pan EC			*Cdh5-CreERT2*	45
			Cldn5-CreERT2	46
			Tie2-Cre	47
			Tmem100-CreERT2	17
	AEC		*Gja5-CreERT2*	48
			Sox17-Cre	49
			Bmx-CreERT2	50
			Cxcl12-CreERT2	51
	Capillary EC	gCap (Cap1)	*Plvap-CreERT2*	52
			Plvap-DreERT2	53
			Kit-CreERT2	54,55
			Aplnr-CreERT2	56
		aCap (Cap2)	*Car4-CreERT2*	52
			Kdr-Cre	57
			Apln-CreERT2	58
			Ednrb-CreERT2	59
	PVEC		*Nr2f2-CreERT2*	60
			Slc6a2-CreERT2	34
			Vwf-CreERT2	61
	LEC		*Thy1-CreERT2*	62
			Prox1-CreERT2	26
			Lyve1-CreERT2	27

aCap indicates aerocyte; AEC, arterial endothelial cell; EC, endothelial cell; gCap, general capillary endothelial cell; LEC, lymphatic endothelial cell; and PVEC, pulmonary venous endothelial cell.

reported that elevated shear stress promotes EndoMT in pulmonary AECs by suppressing expression of the *ERG*. The mechanosensor Piezo1 (piezo-type mechanosensitive ion channel component) is also induced by increased shear stress, activating downstream notch signaling in AECs and contributing to vascular remodeling in PH.[67] In contrast, another Piezo family member, Piezo2, is downregulated in ECs from patients with PAH; EC-specific deletion of Piezo2 exacerbates PH pathogenesis in hypoxia-induced rat models and impairs tube formation and migration of rat pulmonary microvascular ECs (MVECs).[68] In fact, MVECs are generally regarded as distal ECs isolated from the peripheral lung parenchyma.[69] Evidence also points to differential mechanical responses between proximal and distal ECs. Szulcek et al[70] demonstrated delayed shear stress adaptation in MVECs isolated from patients with PAH, but not in proximal AECs from the same lungs, implicating disrupted PECAM-1 (platelet EC adhesion molecule-1) function in MVEC dysfunction. Follow-up studies further revealed interindividual variation in shear stress-responsive gene programs of PAH MVECs, which could be matched to peripheral blood mononuclear cell omics profiles, highlighting potential avenues for noninvasive molecular diagnostics.[71] Collectively, these findings underscore AEC heterogeneity in shear stress responses while pointing toward translational opportunities though questions remain, given that the definition of lung MVEC in the literature is often implicit. Primary MVECs are commonly defined as ECs isolated from the peripheral lung parenchyma after the removal of large vessels and airways.[69,72,73] The tissue is digested into a single-cell suspension, followed by depletion of non-ECs and enrichment of ECs using pan-endothelial markers, typically with anti-CD31 magnetic beads.[74,75] In histological analyses of lung sections, the definition of lung microvessels is variable in terms of vessel diameter.[76–78] MVECs are generally considered ECs labeled with pan EC markers that are located within the gas exchange regions of the lung and not visibly associated with larger arterioles or venules.[78] Consequently, MVECs represent a mixed population of ECs derived from multiple small vessel types, including arterioles, postcapillary venules, and capillaries. This heterogeneous population cannot be directly aligned with any specific EC subpopulation identified by scRNA-seq.

Single-cell RNA sequencing of mouse ECs from Sugen 5416 (an inhibitor of VEGFR)/hypoxia (SuHx)-induced PH (a widely used experimental model combining VEGFR inhibition with chronic hypoxia to induce severe PH[79]) conditions revealed a significant upregulation of the antigen processing and presentation pathway in AEC.[8] This approach, compared with rat and human PAH data sets, identified CD74 as a candidate molecule involved in EC proliferation and barrier integrity.[8] Consistently, another single-cell study in SuHx rats also reported a distinct subset of dedifferentiated ECs enriched for *Cd74*.[80] Dedifferentiated ECs exhibited a primed EndoMT state, including loss of EC identity genes such as Erg, and tight junction components *Cldn5* and *Cdh5* (cadherin 5). In the same study, a novel subset of activated ECs characterized by high expression of *Tm4sf1*, a gene associated with cancer cell growth, was also identified. Longitudinal single-cell profiling across multiple time points in SuHx rats demonstrated early and sustained expansion of both activated ECs and dedifferentiated ECs during PAH initiation and progression.[80] These findings suggest that AECs are not only transcriptionally heterogeneous but also dynamically remodeled under PAH, with subsets adopting immune cell recruitment and EndoMT. Such plasticity highlights the pivotal role of AECs in driving vascular remodeling and barrier dysfunction in the vascular remodeling of PAH. Transcriptomic profiling of human AEC directly from patients with PAH obtained by right heart catheter balloon tips revealed a stable transcriptomic profile

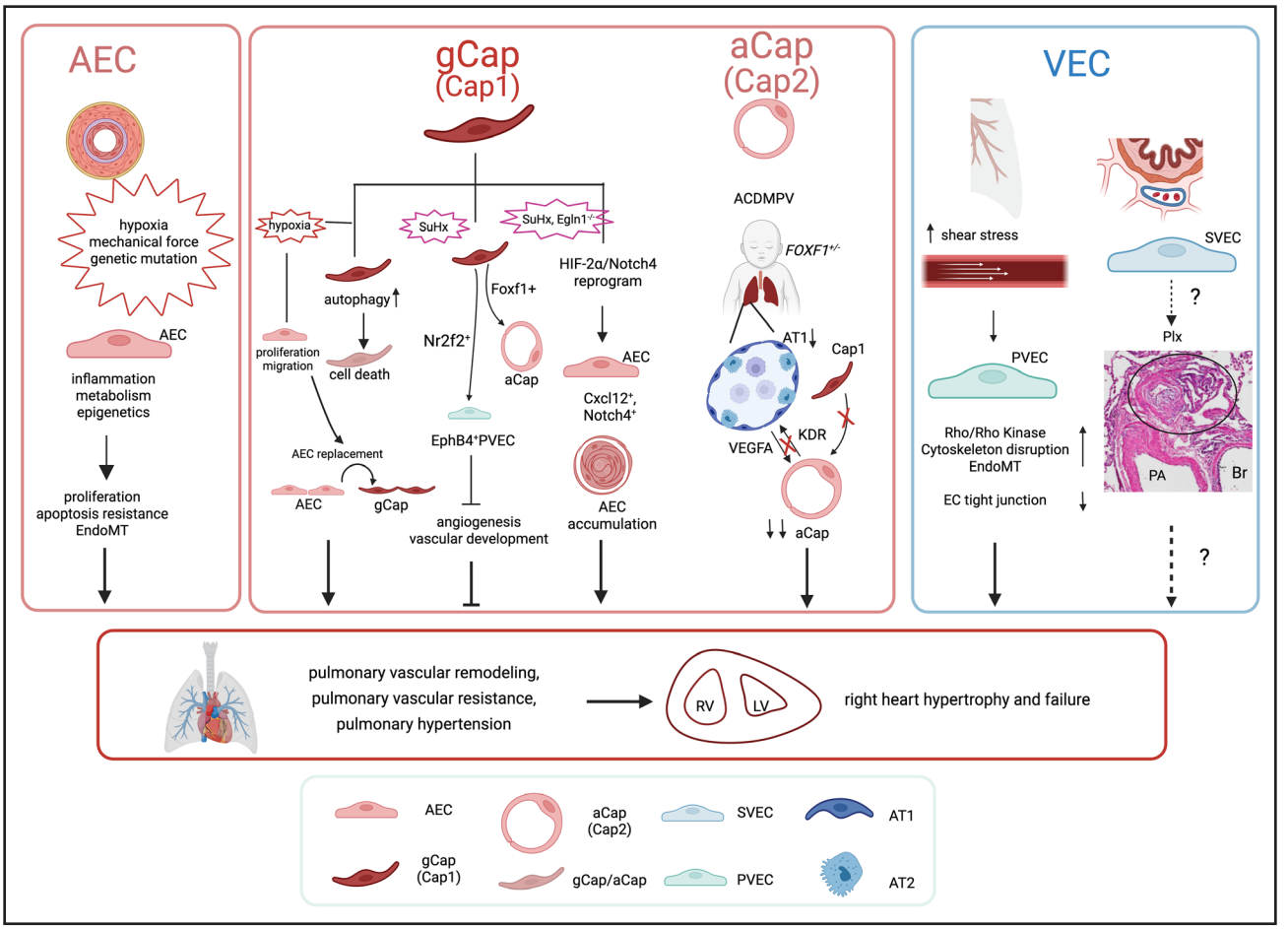

Figure. Pulmonary endothelial cell (EC) dysregulation in pulmonary hypertension (PH).
EC dysregulation in PH has been extensively reviewed elsewhere.[1,6,65] Briefly, environmental stressors, mechanical forces, and genetic perturbations activate abnormal inflammatory, metabolic, and epigenetic responses in arterial ECs (AECs). These changes promote a proliferative, antiapoptotic, and endothelial-mesenchymal transition (EndoMT) phenotype that contributes to vascular remodeling in PH. Hypoxia induces autophagy in capillary (Cap) ECs while promoting AEC proliferation, resulting in AECs' replacement of Cap ECs during vascular remodeling. General Cap ECs (gCaps or Cap1) have also been reported to reprogram into AECs in the Sugen 5416/hypoxia (SuHx)–deficient and *Egln1*-deficient mouse model, a process regulated by the HIF-2α (hypoxia-induced factor-2α)/Notch4 (Notch receptor 4) axis. By contrast, intratracheal delivery of AAV (adeno-asscociated virus)-Nr2f2 (nuclear receptor subfamily 2, group F, member 2) promotes gCaps differentiation into *EphB4*$^{+}$ pulmonary venous ECs (PVECs), suppressing angiogenesis and vascular development, thereby mitigating SuHx-induced PH in mouse models. In alveolar capillary dysplasia with misalignment of pulmonary veins (ACDMPV)-PH, *FOXF1* deficiency impairs gCap differentiation into aCap (aerocyte or Cap2) and compromises aCap survival through reduced VEGFA (vascular endothelial growth factor A)-KDR (kinase insert domain receptor) signaling. In pulmonary veins, group 2 PH is associated with elevated shear stress that activates ρ/ρ-kinase and EndoMT signaling pathways, disrupting PVECs' tight junctions and promoting vascular remodeling. SVECs may serve as a compensatory contributor to pulmonary perfusion under obstructed conditions in PH. The solid lines represent causal connections, while the dotted lines indicate potential connections. The arrows leading to the panel borders suggest that the pathway contributes to the development of PH.

through limited serial passages. Notably, downregulated pathways included the HOP (homeodomain-only protein) pathway, ALK (anaplastic lymphoma kinase) pathway, leukocyte transendothelial migration, and Fanconi anemia pathway in PAH subjects.[81] In PAH, downregulated genes were associated with BMP (bone morphogenetic protein) signaling and WNT signaling. Furthermore, AECs from groups 2 to 5 precapillary PH subjects and PAH exhibited a similar transcriptomic profile, suggesting a potential common role of AECs in vascular remodeling across different PH groups.[81] However, one important caveat is that the in vitro culture environment may lead to the loss of native transcriptomic signatures in AECs. A recent study of single-cell profiling in lung samples of patients with systemic sclerosis–related PH, idiopathic PAH, and healthy controls highlighted significant upregulation of fibrosis pathway (pulmonary fibrosis idiopathic signaling pathway), angiogenesis (hypoxia inducible factor signaling), inflammation (interleukin-8 signaling), and ECM remodeling (matrisome pathway) in AECs of systemic sclerosis–related PH. An overlap of upregulated ECM signaling in AEC between systemic sclerosis–related PH and idiopathic PAH was reported, providing evidence of common features in end-stage PAH.[82]

Capillary EC

The identification of gCaps as progenitor cells that proliferate and replenish capillary ECs[20] has led to important research on their role in endothelium repair during lung injury. Godoy et al[83] showed that a subset of gCaps characterized by expression of apelin rapidly replenished all EC types 7 days after acute lung injury caused by EC depletion in mice. Furthermore, the *ERG* helped maintain the stemness of gCaps,[84] which was based on the evidence that the endothelial-specific *Erg*-deficient mouse lungs showed a reduction of gCaps and vascular remodeling. Notably, gCaps were found to be significantly downregulated in patients with IPF.[84] Additional genes, such as *Atf3*, found in a subset of gCaps, contribute to endothelium regeneration after acute lung injury induced by H1N1 infection in mice.[85] In contrast, an *FOXF1* mutation in gCaps disrupted the BMP9/ACVRL1/ (activin A receptor-like type 1) SMAD1 (sterile alpha motif domain containing 1) pathway, leading to alveolar capillary dysplasia with misalignment of PVs, a severe developmental lung disorder with PH as a common complication.[86]

Despite multiple studies underscoring the importance of gCaps in lung injury repair, the role in PH remains controversial. A recent study from Zhang et al[87] reported that gCaps exhibited distinct responses compared with AECs during the early stage of hypoxia-induced PH in mice. Specifically, gCaps showed enrichment of proapoptotic genes and proangiogenic pathways, whereas AECs generally exhibited an apoptosis-resistant and proliferative phenotype. The authors further demonstrated that chronic hypoxia-induced autophagy promoted AECs proliferation but triggered apoptosis in MVECs, defined as ECs in arterioles <20 μm in diameter and *Griffonia simplicifolia* IB4$^+$/Weibel-Palade bodies$^-$,[73] leading to the replacement of MVECs by AECs in precapillary arterioles at the early stage of hypoxia.[87] However, it is important to note that their histological assessment of MVECs within arterioles may not fully correspond to the transcriptional signatures identified in gCaps by scRNA-seq because gCaps are typically localized within the capillary network rather than arteriolar segments. This distinction suggests that the responses observed histologically in MVECs may not directly reflect the biology of gCaps identified by transcriptional analyses, emphasizing the need for careful consideration of vascular compartmentalization (arteriolar versus capillary) when interpreting these findings. In contrast, Liu et al[53] proposed that PH is characterized by a reduced proportion of gCaps and aCaps and a concomitant expansion of AECs in patients with PAH. This was supported by both genetic lineage tracing studies (*Plvap-DreERT2* mice) and scRNA-seq data, which demonstrated that gCaps can reprogram into AECs in ECs of *Egln1*-deficient mice that develop spontaneous PH.[53] The findings in these 2 studies are not necessarily mutually exclusive but may reflect differences in disease stage, animal models, or the specific vascular compartments (arterioles versus capillaries) examined. Collectively, both studies converge on the concept that AECs enrichment underlies PH pathogenesis, either through phenotypic transition of gCaps or apoptotic loss of MVECs, leading to the accumulation of AECs (Figure).

Adding further complexity to gCaps plasticity, a recent study from Li et al[88] reported that lung resident c-kit^{pos} cells, which are closely associated with the gCap population, have the potential to differentiate into PVECs, aCaps, and AECs. Lineage tracing study in the SuHx mouse model showed that c-kit^{pos} cells expanded and differentiated into mature PVECs and aCaps, while their contribution to the AEC lineage remained relatively unchanged.[88] Ablation of c-kit^{pos} cells exacerbated vascular remodeling and right ventricle hypertrophy. In addition, this study also identified a c-kit^{pos} cell subpopulation with high expression of the venous endothelial marker Nr2f2 in PH. Overexpression of Nr2f2 via adeno-associated virus in c-Kit^{pos} cells promoted their differentiation into PVECs, thereby mitigating vascular remodeling and restoring microvascular homeostasis. The divergent findings regarding the differentiation potential of gCaps into PVECs, aCaps, or AECs may arise from methodological differences, particularly the markers used to define AECs, as well as the differences in PH models used.[53,88] Beyond these technical considerations, local environmental cues that shape gCaps differentiation, the dynamic alteration of EC subpopulations across capillaries and microvessel beds, and the origins of those ECs warrant further investigation.

aCaps represent a highly differentiated and specialized cell population specified by AT1 cell–derived VEGFA, which drives alveolar genesis during development.[24] The importance of VEGFA in aCaps is underscored by findings that endothelial TGF-βR2 (transforming growth factor-β receptor 2) deficiency impaired autocrine VEGFA production, thereby restricting aCaps renewal during acute lung injury.[89] In the early postnatal stage, studies in mouse models of bronchopulmonary dysplasia characterized by vascular rarefaction and alveolar simplification showed that aCaps are particularly vulnerable to hyperoxia-induced damage.[90,91] Reduced aCaps abundance has also been reported in alveolar capillary dysplasia with misalignment of PVs, with a lower proportion correlating with greater disease severity.[92] Although aCaps have limited regenerative capacity, several studies suggest that they play roles in acute injury repair. A recent longitudinal study of acute lung injury identified an injury-induced state of capillary EC, defined by *Sparcl1* and *Ntrk2* expressions, which emerged at sites of injury and derived from both gCap and aCap lineages.[59] Similarly, another study identified Car4^{high} ECs localized to regenerative lesions after acute injury and was primed to receive reparative signals from AT1 cells.[93] In SuHx mouse models, aCap exhibited upregulation of genes involved in cell death, cell motility, and angiogenesis.[8] Because aCaps are considered

terminally differentiated and highly specialized, research has largely focused on gCaps due to their greater regenerative capacity, leaving many aspects of aCaps unresolved. Key knowledge gaps include (1) functional roles of aCaps in gas exchange and leukocyte trafficking, which remain largely inferred from transcriptomic profiles and spatial localization rather than direct functional assays[20]; (2) discrepancies across studies regarding their proliferative capacity and contribution to injury repair; and (3) the status of aCaps in chronic lung diseases such as chronic obstructive lung disease, IPF, and PH, where it remains unclear whether alterations in their abundance or function correlate with disease progression. Addressing these questions will be critical to fully understand the contribution of this unique capillary endothelial subpopulation to lung homeostasis and pathology.

PVEC

Venous ECs are a highly plastic cell population considered a reservoir for sprouting angiogenesis.[33] PVECs have been reported to differentiate into gCaps and aCaps following lung injury, thereby contributing to vascular bed repair.[34] In a porcine model of group 2 PH, PV remodeling was characterized by marked increases in smooth muscle cells and increased fibrosis. Proteomics analysis showed that pathways related to EndoMT and endothelial barrier disruption were more significant in remodeled PV rather than PA, suggesting that VECs are more sensitive to stress in group 2 PH.[94] Additional evidence links PVEC dysregulation to aging-associated lung disease.[95] Raslan et al[95] demonstrated that Ackr1^{pos} VECs were activated and expanded in an aged lung fibrosis mouse model, exhibiting enrichment of hypoxia, glycolysis, and YAP/TAZ (Yes-associated protein/tracriptional coactivator with PDZ-binding motif) signaling, along with inflammatory and angiogenic states. In PH, PVECs also exhibited upregulation of antigen processing pathways, a finding that is not exclusive to PVECs and is observed in other cell types.[8] Histological evidence has shown that a proportion of PAH and chronic thromboembolic PH lungs exhibited muscularization of PVs.[96] It is worth noting the role of PVEC in vascular remodeling in PH, given their involvement in inflammation and their pronounced plasticity. Targeting PVEC dysregulation may represent a promising therapeutic strategy to slow PH progression and mitigate disease severity in a specific subset of patients.

SVEC

The human lung is unique in receiving blood from 2 circulatory systems. The pulmonary circulation delivers deoxygenated blood to the alveolar capillary network for gas exchange, while the bronchial circulation, originating from the systemic circulation, supplies oxygenated blood to the main bronchi, large pulmonary arteries, and visceral pleura.[97] In addition, the normal bronchial circulation forms extensive anastomoses with, and drains into, the pulmonary venous circulation, creating a small, physiological shunt under normal conditions. The role of SVECs under pathological conditions remains less well understood. A study in alveolar capillary dysplasia with misalignment of PVs, a lethal developmental lung disorder characterized by PH and hypertensive remodeling of pulmonary arteries,[98] observed that marked expansion of SVECs has been observed, accompanied by enhanced VEGFA signaling from AT1/alveolar type II epithelial cells to SVECs.[92] These findings suggest a possible compensatory role of SVECs in the setting of impaired pulmonary circulatory perfusion. In adult PAH, studies of vascular lesion subtypes have proposed that the systemic circulation contributes to the formation of the plexiform lesion, a hallmark of severe PAH composed of proliferating ECs, smooth muscle cells, and other cell types, through bronchopulmonary shunts that arise under high pressure within the pulmonary circulation.[96,99–101] These findings implicate SVECs as potential contributors to pathological vascular remodeling in PAH. Nevertheless, it remains unclear whether the penetration of systemic circulation reflects an early compensatory adaptation during the onset of PAH or a maladaptive response that accelerates disease progression.

LEC

Lung lymphatic vessels are critical for immune surveillance and fluid homeostasis. The role of lung lymphatic vessels is widely studied in lung transplantation, where the disruption of the normal structure of lymphatic vessels and the pulmonary lymphatic drainage leads to graft rejection.[102–104] However, the role of lymphatic vessels in PH and chronic lung diseases remains less well understood. Several studies have highlighted the remodeling of lymphatic vessels in IPF, including abnormal lymphangiogenesis,[105,106] aberrant recruitment of mural cells,[107] and accumulation of fibroblasts.[107] Using a bleomycin-induced lung injury model, Meinecke et al[107] revealed that PDGF-B in LEC and PDGFR-β signaling in mural cells drove the lymphatic remodeling process at the early stage of fibrosis. Pharmacological inhibition of PDGF-B/PDGFR-β signaling improved lymphatic drainage and prevented fibroblast recruitment to the perilymphatic niche.[107] A single-cell atlas of LECs revealed the heterogeneity within this population. A Pdl1^{pos} subset of LECs was potentially responsible for lymphatic vessel remodeling.[108] In addition, LECs showed increased inflammatory and prothrombotic signaling in patients with chronic obstructive pulmonary disease.[94] Perivascular inflammation is one of the prominent features of PAH. The future direction for LEC in PH could be focused on inflammatory regulation and cell communication with vascular ECs in disease progression.

EC-TARGETED NANOMEDICINE IN PH

Canonical therapeutic interventions for PH, particularly PAH, remain inadequate in substantially reducing mortality, in part due to limitations such as drug instability, poor solubility, and systemic side effects.[7,109] Nanoparticle-based delivery systems offer a promising strategy to overcome these challenges. Nanomaterials can be engineered to target specific regions, enhancing therapeutic precision.[110,111] Given that ECs are directly exposed to blood flow and lack a protective mucus barrier, intravenously administered nanoparticles can efficiently reach them, with uptake modulated by hemodynamic forces.[112] EC-targeted nanomedicine, particularly for gene therapy, represents an emerging and innovative therapeutic approach for PH.

Several studies have demonstrated the therapeutic potential of EC-targeted nanomedicine in PH. Intravenous nanoparticle delivery of CMV-Stat3 (cytomegalovirus/signaling transducer and activator of transcription 3) plasmid DNA at postnatal day 2 protected *Foxf1*$^{WT/S52F}$ mice, a mouse model with AMCDPV and PH, from PH and RV hypertrophy, and improved survival rate.[113] In addition, Liu et al[114] achieved efficient knockdown of *Nos3* in ECs using nanoparticle-delivered CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9) driven by the CDH5 promoter, which significantly improved vascular remodeling and hemodynamic parameters in *Egln1*^{Tie2cre} mice, a severe PH model. Interestingly, Yuan et al[115] developed a newly edited nanoparticle, ACE2-CS-PRT@PM, containing hypoxia-responsive plasmid ACE2 driven by Tie2 (TEK [TEK receptor tyrosine kinase] receptor tyrosine kinase) promoter, which enabled ACE2 overexpression only under hypoxic conditions. Intravenous delivery of ACE2-CS-PRT@PM (angiotensin-converting enzyme 2-chondroitin sulfate-protamine-platelet membrane) ameliorated the hemodynamic dysfunction in hypoxic PH rats.

The current nanodelivery system demonstrates effective uptake in lung ECs. However, complete avoidance of ECs uptake in other organs remains a challenge. This limitation is partly due to the use of pan-endothelial promoters or targeting drivers such as CDH5 and TEK, which lack organ specificity. Recently, TMEM100 (transmembrane protein 100) has been identified as a lung-specific endothelial marker, offering a promising avenue for improving targeting specificity in future delivery systems.[14,17] In addition, the advances in scRNA-seq have brought research in PH to a higher resolution, further highlighting the urgent need for specific delivery systems capable of targeting distinct vascular compartments while minimizing off-target effects. Deng et al[116] developed a fluorinated amphiphilic poly (β-amino ester) nanoparticle with specific design and fluorinated modification that specifically targets pulmonary MVECs rather than other cell types in the lung, making it a promising delivery system for pulmonary microvasculature.

Collectively, these advances highlight the potential of EC-targeted nanomedicine to precisely modulate gene expression within the pulmonary vasculature. Such strategies represent a promising direction for developing more effective and personalized therapies for PH.

ADVANCED TECHNOLOGIES IN PH RESEARCH

Multiple aspects of EC dysregulation, including alterations in metabolism, inflammation, epigenetic modifications, EndoMT, and crosstalk with other cell types, contribute to the initiation and progression of PH. Recent advances in cutting-edge technologies are deepening mechanistic insights and facilitating the identification of novel diagnostic and therapeutic targets.

Single-cell technologies have revolutionized our understanding of lung EC heterogeneity in both healthy and diseased conditions. However, a major limitation of scRNA-seq is the loss of spatial context during tissue dissociation.[117] Spatial transcriptomics, by preserving tissue architecture, complements single-cell approaches by enabling the identification of region-specific transcriptional programs and mapping cell-cell interactions that contribute to vascular remodeling and endothelial dysfunction in PH.[117,118] Employing Visium spatial transcriptomics, Liu et al,[53,119] demonstrated that in the wild-type lung, AECs were found primarily in proximal regions, and gCaps were detected in distal microvascular regions. In contrast, *Egln1*^{Tie2Cre} mice lungs exhibited an increase in AECs and a decrease in gCaps and aCaps signatures in the distal microvascular bed.[53,119] These data partly support the transition of gCaps to AECs in the distal microvascular bed in PH. However, each spot (55 μm) in the Visium slide typically captures the transcriptomic data from a small population of cells, rather than a single cell. It is difficult to resolve the distinct transcriptomic signatures of neighboring but functionally different cell types like alveoli. The hallmark vasculopathy of PAH is characterized by the plexiform and obliterative lesions, intimal and medial hypertrophy, and adventitial remodeling. Using digital spatial profiling, Tuder et al[120] provided comprehensive insight into the cellular and molecular features of different vascular lesions in PAH. Their findings revealed that both the cellular composition and transcriptomic signatures varied significantly among different lesion types. ECs were most prominently enriched in plexiform lesions, where dysregulated pathways involved angiogenesis and hypoxia. Smooth muscle cells were prominent in the obliterative lesion and regions of intimal and medial hypertrophy, while fibroblasts and myofibroblasts were enriched in adventitial changes. However, Tuder et al used a region-of-interest–based bulk expression profiling approach rather than mapping transcripts to vascular lesions at cellular or subcellular resolution. Cell-type

proportions were inferred from the relative abundance of marker gene transcripts (ie, changes in CDH5, CLDN5, and PECAM-1 were used to indicate EC alterations). One limitation of this strategy is the lack of specificity in cell identification because marker genes are often shared across multiple cell types, bulk averaging obscures cellular heterogeneity, and the lack of single-cell resolution prevents precise spatial assignment of transcripts to specific vascular or stromal compartments. Furthermore, using fixed frozen mouse lung sections with free-floating mounting onto single-cell resolution Xenium slides, Zhao et al[121] identified 40 major lung cell types and identified key cellular changes including an increase in AECs and fibroblasts and a reduction of aCaps in PH lung. Although the resolution of spatial transcriptomics has improved to the subcellular level, important limitations remain. High-resolution platforms are typically restricted to a targeted set of genes, whereas whole-transcriptome platforms sacrifice resolution,[122] potentially limiting the identification of rare or specific cell subtypes, particularly within the complex pulmonary vascular niche. Moreover, robust computational frameworks and standardized analytical pipelines are still needed to enhance reproducibility and facilitate cross-study comparisons.

Transcriptional analyses have shown that in vitro cultivation can lead to a loss of EC heterogeneity, limiting the ability to faithfully model physiological and pathological states.[12] Moreover, the lack of available primary lung capillary EC culture systems presents a major challenge for studying human capillary EC function and behavior under PH conditions in vitro. Human precision-cut lung slices derived from patient lungs represent a promising strategy. Precision-cut lung slices are prepared from fresh lung tissue using a vibratome or other tissue slicer, typically at a thickness of 150 to 500 µm,[123] which allows for better preservation of alveolar, vascular, and interstitial structures.[124,125] In PH research, precision-cut lung slices can be generated from distinct anatomic regions of diseased lung, such as proximal versus distal areas affected by disease. This enables the investigation of region-specific endothelial phenotypes and vascular remodeling. One important consideration is that precision-cut lung slices typically are derived from explanted tissue with an end-stage disease state.[124] Vascular organoids derived from patient-specific induced pluripotent stem cells provide another promising approach for studying microvasculature and capillary involvement in the initiation and progression of PH. Miao et al[126] recently developed a human induced pluripotent stem cell–derived organoid system comprising lung epithelium surrounded by organotypic mesenchyme and vasculature. These organoids generated perfusable human-specific capillaries upon kidney capsule transplantation in mice. The team later demonstrated the ability of these organoids to model alveolar capillary dysplasia with alveolar capillary dysplasia with misalignment of PVs using induced pluripotent stem cells derived from affected patients.[126] These advances provide a powerful platform to study PH pathogenesis in vitro, particularly for PH arising from congenital developmental disorders.

CONCLUSIONS

Advances in understanding EC heterogeneity have fundamentally reshaped our view of the complexity of PH. As distinct molecular profiles, cellular interactions, and functional roles of diverse EC subtypes continue to be uncovered, we are moving closer to fully deciphering the mechanisms driving PH and advancing toward more precise diagnostic and therapeutic strategies. Future research should focus on characterizing EC states at the early stages of disease and identifying transitional phenotypes during disease progression. Spatial omics technologies provide valuable insights into the molecular architecture of vasculopathy, enabling the study of cell-cell communication and the immune microenvironment within a spatial context. In addition, patient-specific drug screening using induced pluripotent stem cell–derived ECs offers a promising avenue for translational research, bridging the gap between molecular discovery and clinical application. Ultimately, integrating cutting-edge technologies with functional studies and patient-derived models will pave the way for a more comprehensive and personalized approach to understanding and treating PH.

ARTICLE INFORMATION

Affiliations

Division of Pulmonary and Critical Care Medicine, Department of Medicine, Washington University School of Medicine in St. Louis, MO (H.Z., M.M.C., S.M.L., J.S.L., Z.D.). Department of Internal Medicine, College of Medicine-Phoenix, The University of Arizona, Phoenix (M.B.F.).

Author Contributions

H. Zhao drafted the manuscript. M.M. Chakinala, M.B. Fallon, S.M. Lin, J.S. Lee, and Z. Dai revised the manuscript.

Sources of Funding

This work was supported in part by the National Institutes of Health grants R01HL158596, R01HL162794, R01HL169509, and R01HL170096 and Washington University institution funding to Z. Dai.

Disclosures

None.

REFERENCES

1. Mocumbi A, Humbert M, Saxena A, Jing Z-C, Sliwa K, Thienemann F, Archer SL, Stewart S. Pulmonary hypertension. *Nat Rev Dis Primers*. 2024;10:1. doi: 10.1038/s41572-023-00486-7
2. Guignabert C, Aman J, Bonnet S, Dorfmüller P, Olschewski AJ, Pullamsetti S, Rabinovitch M, Schermuly RT, Humbert M, Stenmark KR. Pathology and pathobiology of pulmonary hypertension: current insights and future directions. *Eur Respir J*. 2024;64:2401095. doi: 10.1183/13993003.01095-2024
3. Rafii S, Butler JM, Ding B-S. Angiocrine functions of organ-specific endothelial cells. *Nature*. 2016;529:316–325. doi: 10.1038/nature17040
4. Sharma A, Niethamer TK. Specialized pulmonary vascular cells in development and disease. *Annu Rev Physiol*. 2025;87:229–255. doi: 10.1146/annurev-physiol-022724-105226

5. Flammer AJ, Anderson T, Celermajer DS, Creager MA, Deanfield J, Ganz P, Hamburg NM, Lüscher TF, Shechter M, Taddei S, et al. The assessment of endothelial function: from research into clinical practice. *Circulation*. 2012;126:753–767. doi: 10.1161/CIRCULATIONAHA.112.093245
6. Evans CE, Cober ND, Dai Z, Stewart DJ, Zhao Y-Y. Endothelial cells in the pathogenesis of pulmonary arterial hypertension. *Eur Respir J*. 2021;58:2003957. doi: 10.1183/13993003.03957-2020
7. Ghofrani H-A, Gomberg-Maitland M, Zhao L, Grimminger F. Mechanisms and treatment of pulmonary arterial hypertension. *Nat Rev Cardiol*. 2025;22:105–120. doi: 10.1038/s41569-024-01064-4
8. Rodor J, Chen SH, Scanlon JP, Monteiro JP, Caudrillier A, Sweta S, Stewart KR, Shmakova A, Dobie R, Henderson BEP, et al. Single-cell RNA sequencing profiling of mouse endothelial cells in response to pulmonary arterial hypertension. *Cardiovasc Res*. 2022;118:2519–2534. doi: 10.1093/cvr/cvab296
9. Asosingh K, Comhair S, Mavrakis L, Xu W, Horton D, Taylor I, Tkachenko S, Hu B, Erzurum S. Single-cell transcriptomic profile of human pulmonary artery endothelial cells in health and pulmonary arterial hypertension. *Sci Rep*. 2021;11:14714. doi: 10.1038/s41598-021-94163-y
10. Crnkovic S, Valzano F, Fließer E, Gindlhuber J, Thekkekara Puthenparampil H, Basil M, Morley MP, Katzen J, Gschwandtner E, Klepetko W, et al. Single-cell transcriptomics reveals skewed cellular communication and phenotypic shift in pulmonary artery remodeling. *JCI Insight*. 2022;7:e153471. doi: 10.1172/jci.insight.153471
11. Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. Cell number and cell characteristics of the normal human lung. *Am Rev Respir Dis*. 1982;126:332–337. doi: 10.1164/arrd.1982.126.2.332
12. Schupp JC, Adams TS, Cosme C, Raredon MSB, Yuan Y, Omote N, Poli S, Chioccioli M, Rose K-A, Manning EP, et al. Integrated single-cell atlas of endothelial cells of the human lung. *Circulation*. 2021;144:286–302. doi: 10.1161/CIRCULATIONAHA.120.052318
13. Kalucka J, De Rooij LPMH, Goveia J, Rohlenova K, Dumas SJ, Meta E, Conchinha NV, Taverna F, Teuwen L-A, Veys K, et al. Single-cell transcriptome atlas of murine endothelial cells. *Cell*. 2020;180:764–779.e20. doi: 10.1016/j.cell.2020.01.015
14. Paik DT, Tian L, Williams IM, Rhee S, Zhang H, Liu C, Mishra R, Wu SM, Red-Horse K, Wu JC. Single-cell RNA sequencing unveils unique transcriptomic signatures of organ-specific endothelial cells. *Circulation*. 2020;142:1848–1862. doi: 10.1161/CIRCULATIONAHA.119.041433
15. Du Y, Ouyang W, Kitzmiller JA, Guo M, Zhao S, Whitsett JA, Xu Y. Lung gene expression analysis web portal version 3: lung-at-a-glance. *Am J Respir Cell Mol Biol*. 2021;64:146–149. doi: 10.1165/rcmb.2020-0308LE
16. Gaddis N, Fortriede J, Guo M, Bardes EE, Kouril M, Tabar S, Burns K, Ardini-Poleske ME, Loos S, Schnell D, et al. LungMAP portal ecosystem: systems-level exploration of the lung. *Am J Respir Cell Mol Biol*. 2024;70:129–139. doi: 10.1165/rcmb.2022-0165OC
17. Liu B, Yi D, Yu Z, Pan J, Ramirez K, Li S, Wang T, Glembotski CC, Fallon MB, Oh SP, et al. TMEM100, a lung-specific endothelium gene. *Arterioscler Thromb Vasc Biol*. 2022;42:1495–1497. doi: 10.1161/ATVBAHA.122.317683
18. Sikkema L, Ramírez-Suástegui C, Strobl DC, Gillett TE, Zappia L, Madissoon E, Markov NS, Zaragosi L-E, Ji Y, Ansari M, et al; Lung Biological Network Consortium. An integrated cell atlas of the lung in health and disease. *Nat Med*. 2023;29:1563–1577. doi: 10.1038/s41591-023-02327-2
19. Travaglini KJ, Nabhan AN, Penland L, Sinha R, Gillich A, Sit RV, Chang S, Conley SD, Mori Y, Seita J, et al. A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature*. 2020;587:619–625. doi: 10.1038/s41586-020-2922-4
20. Gillich A, Zhang F, Farmer CG, Travaglini KJ, Tan SY, Gu M, Zhou B, Feinstein JA, Krasnow MA, Metzger RJ. Capillary cell-type specialization in the alveolus. *Nature*. 2020;586:785–789. doi: 10.1038/s41586-020-2822-7
21. De Rooij LPMH, Becker LM, Teuwen L-A, Boeckx B, Jansen S, Feys S, Verleden S, Liesenborghs L, Stalder AK, Libbrecht S, et al. The pulmonary vasculature in lethal COVID-19 and idiopathic pulmonary fibrosis at single-cell resolution. *Cardiovasc Res*. 2023;119:520–535. doi: 10.1093/cvr/cvac139
22. Wang MM, Zhang X, Lee SJ, Maripudi S, Keep RF, Johnson AM, Stamatovic SM, Andjelkovic AV. Expression of periaxin (PRX) specifically in the human cerebrovascular system: PDZ domain-mediated strengthening of endothelial barrier function. *Sci Rep*. 2018;8:10042. doi: 10.1038/s41598-018-28190-7
23. Li Q, Zhu Z, Wang L, Lin Y, Fang H, Lei J, Cao T, Wang G, Dang E. Single-cell transcriptome profiling reveals vascular endothelial cell heterogeneity in human skin. *Theranostics*. 2021;11:6461–6476. doi: 10.7150/thno.54917
24. Vila Ellis L, Cain MP, Hutchison V, Flodby P, Crandall ED, Borok Z, Zhou B, Ostrin EJ, Wythe JD, Chen J. Epithelial Vegfa specifies a distinct endothelial population in the mouse lung. *Dev Cell*. 2020;52:617–630.e6. doi: 10.1016/j.devcel.2020.01.009
25. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, Chu SG, Raby BA, Deluliis G, Januszyk M, et al. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv*. 2020;6:eaba1983. doi: 10.1126/sciadv.aba1983
26. Srinivasan RS, Dillard ME, Lagutin OV, Lin F-J, Tsai S, Tsai M-J, Samokhvalov IM, Oliver G. Lineage tracing demonstrates the venous origin of the mammalian lymphatic vasculature. *Genes Dev*. 2007;21:2422–2432. doi: 10.1101/gad.1588407
27. Connor AL, Kelley PM, Tempero RM. Lymphatic endothelial lineage assemblage during corneal lymphangiogenesis. *Lab Invest*. 2016;96:270–282. doi: 10.1038/labinvest.2015.147
28. Markewitz BA, Kohan DE, Michael JR. Endothelin-1 synthesis, receptors, and signal transduction in alveolar epithelium: evidence for an autocrine role. *Am J Physiol*. 1995;268:L192–L200. doi: 10.1152/ajplung.1995.268.2.L192
29. Galié N, Manes A, Branzi A. The endothelin system in pulmonary arterial hypertension. *Cardiovasc Res*. 2004;61:227–237. doi: 10.1016/j.cardiores.2003.11.026
30. Schupp JC, Manning EP, Chioccioli M, Kamp JC, Christian L, Ryu C, Herzog E, Kühnel MP, Prasse A, Kaminski N, et al; Yale-MHH-MGH Study Group. Alveolar vascular remodeling in nonspecific interstitial pneumonia: replacement of normal lung capillaries with *COL15A1*-positive endothelial cells. *Am J Respir Crit Care Med*. 2023;208:819–822. doi: 10.1164/rccm.202303-0544LE
31. Lang NJ, Gote-Schniering J, Porras-Gonzalez D, Yang L, De Sadeleer LJ, Jentzsch RC, Shitov VA, Zhou S, Ansari M, Agami A, et al. Ex vivo tissue perturbations coupled to single-cell RNA-seq reveal multilineage cell circuit dynamics in human lung fibrogenesis. *Sci Transl Med*. 2023;15:eadh0908. doi: 10.1126/scitranslmed.adh0908
32. Bosma EK, van Noorden CJF, Schlingemann RO, Klaassen I. The role of plasmalemma vesicle-associated protein in pathological breakdown of blood-brain and blood-retinal barriers: potential novel therapeutic target for cerebral edema and diabetic macular edema. *Fluids Barriers CNS*. 2018;15:24. doi: 10.1186/s12987-018-0109-2
33. Lee H-W, Xu Y, He L, Choi W, Gonzalez D, Jin S-W, Simons M. Role of venous endothelial cells in developmental and pathologic angiogenesis. *Circulation*. 2021;144:1308–1322. doi: 10.1161/CIRCULATIONAHA.121.054071
34. Wong J, Zhao G, Adams-Tzivelekidis S, Wen H, Chandrasekaran P, Michki SN, Gentile ME, Singh M, Kass-Gergi S, Mendoza M, et al. Dynamic behavior and lineage plasticity of the pulmonary venous endothelium. *Nat Cardiovasc Res*. 2024;3:1584–1600. doi: 10.1038/s44161-024-00573-2
35. Adams RH, Alitalo K. Molecular regulation of angiogenesis and lymphangiogenesis. *Nat Rev Mol Cell Biol*. 2007;8:464–478. doi: 10.1038/nrm2183
36. Yoshimatsu Y, Miyazaki H, Watabe T. Roles of signaling and transcriptional networks in pathological lymphangiogenesis. *Adv Drug Deliv Rev*. 2016;99:161–171. doi: 10.1016/j.addr.2016.01.020
37. Maisel K, Outtz Reed H. The lymphatic vasculature in lung homeostasis and disease. *Annu Rev Physiol*. 2025;87:421–446. doi: 10.1146/annurev-physiol-022724-105311
38. Oliver G, Kipnis J, Randolph GJ, Harvey NL. The lymphatic vasculature in the 21st century: novel functional roles in homeostasis and disease. *Cell*. 2020;182:270–296. doi: 10.1016/j.cell.2020.06.039
39. Jurisic G, Maby-El Hajjami H, Karaman S, Ochsenbein AM, Alitalo A, Siddiqui SS, Ochoa Pereira C, Petrova TV, Detmar M. An unexpected role of semaphorin3a-neuropilin-1 signaling in lymphatic vessel maturation and valve formation. *Circ Res*. 2012;111:426–436. doi: 10.1161/CIRCRESAHA.112.269399
40. Bouvrée K, Brunet I, Del Toro R, Gordon E, Prahst C, Cristofaro B, Mathivet T, Xu Y, Soueid J, Fortuna V, et al. Semaphorin3A, Neuropilin-1, and PlexinA1 are required for lymphatic valve formation. *Circ Res*. 2012;111:437–445. doi: 10.1161/CIRCRESAHA.112.269316
41. Worthington AK, Cool T, Poscablo DM, Hussaini A, Beaudin AE, Forsberg EC. IL7Rα, but not Flk2, is required for hematopoietic stem cell reconstitution of tissue-resident lymphoid cells. *Development*. 2022;149:dev200139. doi: 10.1242/dev.200139
42. Mai HL, Nguyen TVH, Bouchaud G, Henrio K, Cheminant M-A, Magnan A, Brouard S. Targeting the interleukin-7 receptor alpha by an anti-CD127 monoclonal antibody improves allergic airway inflammation in mice. *Clin Exp Allergy*. 2020;50:824–834. doi: 10.1111/cea.13665
43. Ramachandran P, Dobie R, Wilson-Kanamori JR, Dora EF, Henderson BEP, Luu NT, Portman JR, Matchett KP, Brice M, Marwick JA, et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. *Nature*. 2019;575:512–518. doi: 10.1038/s41586-019-1631-3

44. Verloop MC. On the arteriae bronchiales and their anastomosing with the arteria pulmonalis in some rodents; a micro-anatomical study. *Acta Anat (Basel).* 1949;7:1–32. doi: 10.1159/000140373
45. Monvoisin A, Alva JA, Hofmann JJ, Zovein AC, Lane TF, Iruela-Arispe ML. VE-cadherin-CreERT2 transgenic mouse: a model for inducible recombination in the endothelium. *Dev Dyn.* 2006;235:3413–3422. doi: 10.1002/dvdy.20982
46. Korecki AJ, Hickmott JW, Lam SL, Dreolini L, Mathelier A, Baker O, Kuehne C, Bonaguro RJ, Smith J, Tan C-V, et al. Twenty-seven tamoxifen-inducible iCre-driver mouse strains for eye and brain, including seventeen carrying a new inducible-first constitutive-ready allele. *Genetics.* 2019;211:1155–1177. doi: 10.1534/genetics.119.301984
47. Kisanuki YY, Hammer RE, Miyazaki J, Williams SC, Richardson JA, Yanagisawa M. Tie2-Cre transgenic mice: a new model for endothelial cell-lineage analysis in vivo. *Dev Biol.* 2001;230:230–242. doi: 10.1006/dbio.2000.0106
48. Matsushita Y, Nagata M, Kozloff KM, Welch JD, Mizuhashi K, Tokavanich N, Hallett SA, Link DC, Nagasawa T, Ono W, et al. A Wnt-mediated transformation of the bone marrow stromal cell identity orchestrates skeletal regeneration. *Nat Commun.* 2020;11:332. doi: 10.1038/s41467-019-14029-w
49. Liao WP, Uetzmann L, Burtscher I, Lickert H. Generation of a mouse line expressing Sox17-driven Cre recombinase with specific activity in arteries. *Genesis.* 2009;47:476–483. doi: 10.1002/dvg.20520
50. Fowler JL, Zheng SL, Nguyen A, Chen A, Xiong X, Chai T, Chen JY, Karigane D, Banuelos AM, Niizuma K, et al. Lineage-tracing hematopoietic stem cell origins in vivo to efficiently make human HLF+ HOXA+ hematopoietic progenitors from pluripotent stem cells. *Dev Cell.* 2024;59:1110–1131.e22. doi: 10.1016/j.devcel.2024.03.003
51. Noels H, Zhou B, Tilstam PV, Theelen W, Li X, Pawig L, Schmitz C, Akhtar S, Simsekyilmaz S, Shagdarsuren E, et al. Deficiency of endothelial Cxcr4 reduces reendothelialization and enhances neointimal hyperplasia after vascular injury in atherosclerosis-prone mice. *Arterioscler Thromb Vasc Biol.* 2014;34:1209–1220. doi: 10.1161/ATVBAHA.113.302878
52. Zhang Z, Zhou B. Generation of Plvap-CreER and Car4-CreER for genetic targeting of distinct lung capillary populations. *J Genet Genomics.* 2022;49:1093–1100. doi: 10.1016/j.jgg.2022.08.001
53. Liu B, Yi D, Xia X, Ramirez K, Zhao H, Cao Y, Tripathi A, Dong R, Gao A, Ding H, et al. General capillary endothelial cells undergo reprogramming into arterial endothelial cells in pulmonary hypertension through HIF-2α/Notch4 pathway. *Circulation.* 2024;150:414–417. doi: 10.1161/CIRCULATIONAHA.123.067981
54. Liu Q, Huang X, Zhang H, Tian X, He L, Yang R, Yan Y, Wang Q-D, Gillich A, Zhou B. c-kit(+) cells adopt vascular endothelial but not epithelial cell fates during lung maintenance and repair. *Nat Med.* 2015;21:866–868. doi: 10.1038/nm.3888
55. van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin S-CJ, Middleton RC, Marbán E, Molkentin JD. c-kit+ cells minimally contribute cardiomyocytes to the heart. *Nature.* 2014;509:337–341. doi: 10.1038/nature13309
56. Chen HI, Poduri A, Numi H, Kivela R, Saharinen P, McKay AS, Raftrey B, Churko J, Tian X, Zhou B, et al. VEGF-C and aortic cardiomyocytes guide coronary artery stem development. *J Clin Invest.* 2014;124:4899–4914. doi: 10.1172/JCI77483
57. Licht AH, Raab S, Hofmann U, Breier G. Endothelium-specific Cre recombinase activity in flk-1-Cre transgenic mice. *Dev Dyn.* 2004;229:312–318. doi: 10.1002/dvdy.10416
58. Liu Q, Hu T, He L, Huang X, Tian X, Zhang H, He L, Pu W, Zhang L, Sun H, et al. Genetic targeting of sprouting angiogenesis using Apln-CreER. *Nat Commun.* 2015;6:6020. doi: 10.1038/ncomms7020
59. Niethamer TK, Planer JD, Morley MP, Babu A, Zhao G, Basil MC, Cantu E, Frank DB, Diamond JM, Nottingham AN, et al. Longitudinal single-cell profiles of lung regeneration after viral infection reveal persistent injury-associated cell states. *Cell Stem Cell.* 2025;32:302–321.e6. doi: 10.1016/j.stem.2024.12.002
60. Brown PR, Estermann MA, Gruzdev A, Scott GJ, Hagler TB, Ray MK, Yao HH-C. Generation of a Nr2f2-driven inducible Cre mouse to target interstitial cells in the reproductive system. *Biol Reprod.* 2025;113:220–230. doi: 10.1093/biolre/ioaf094
61. Yadav D, Conner JA, Wang Y, Saunders TL, Ubogu EE. A novel inducible von Willebrand factor Cre recombinase mouse strain to study microvascular endothelial cell-specific biological processes in vivo. *Vascul Pharmacol.* 2024;155:107369. doi: 10.1016/j.vph.2024.107369
62. Young P, Qiu L, Wang D, Zhao S, Gross J, Feng G. Single-neuron labeling with inducible Cre-mediated knockout in transgenic mice. *Nat Neurosci.* 2008;11:721–728. doi: 10.1038/nn.2118
63. Kovacs G, Bartolome S, Denton CP, Gatzoulis MA, Gu S, Khanna D, Badesch D, Montani D. Definition, classification and diagnosis of pulmonary hypertension. *Eur Respir J.* 2024;64:2401324. doi: 10.1183/13993003.01324-2024
64. Kostyunina DS, Rowan SC, Pakhomov NV, Dillon E, Rochfort KD, Cummins PM, O'Rourke MJ, McLoughlin P. Shear stress markedly alters the proteomic response to hypoxia in human pulmonary endothelial cells. *Am J Respir Cell Mol Biol.* 2023;68:551–565. doi: 10.1165/rcmb.2022-0340OC
65. Cober ND, VandenBroek MM, Ormiston ML, Stewart DJ. Evolving concepts in endothelial pathobiology of pulmonary arterial hypertension. *Hypertension.* 2022;79:1580–1590. doi: 10.1161/HYPERTENSIONAHA.122.18261
66. Shinohara T, Moonen J-R, Chun YH, Lee-Yow YC, Okamura K, Szafron JM, Kaplan J, Cao A, Wang L, Guntur D, et al. High shear stress reduces ERG causing endothelial-mesenchymal transition and pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol.* 2025;45:218–237. doi: 10.1161/ATVBAHA.124.321092
67. Wang Z, Chen J, Babicheva A, Jain PP, Rodriguez M, Ayon RJ, Ravellette KS, Wu L, Balistrieri F, Tang H, et al. Endothelial upregulation of mechanosensitive channel Piezo1 in pulmonary hypertension. *Am J Physiol Cell Physiol.* 2021;321:C1010–C1027. doi: 10.1152/ajpcell.00147.2021
68. Wei F, Lin Z, Lu W, Luo H, Feng H, Liu S, Zhang C, Zheng Y, Chen J, Mo S, et al. Deficiency of endothelial Piezo2 impairs pulmonary vascular angiogenesis and predisposes pulmonary hypertension. *Hypertension.* 2025;82:583–597. doi: 10.1161/HYPERTENSIONAHA.124.22948
69. Hewett PW, Murray JC. Human lung microvessel endothelial cells: isolation, culture, and characterization. *Microvasc Res.* 1993;46:89–102. doi: 10.1006/mvre.1993.1037
70. Szulcek R, Happé CM, Rol N, Fontijn RD, Dickhoff C, Hartemink KJ, Grünberg K, Tu L, Timens W, Nossent GD, et al. Delayed microvascular shear adaptation in pulmonary arterial hypertension. Role of platelet endothelial cell adhesion molecule-1 cleavage. *Am J Respir Crit Care Med.* 2016;193:1410–1420. doi: 10.1164/rccm.201506-1231OC
71. Wittig C, König JM, Pan X, Aman J, Bogaard H-J, Yu PB, Kuebler WM, Baum K, Szulcek R. Shear stress unveils patient-specific transcriptional signatures in PAH: towards personalized molecular diagnostics. *Theranostics.* 2025;15:1589–1605. doi: 10.7150/thno.105729
72. Lou JN, Mili N, Decrind C, Donati Y, Kossodo S, Spiliopoulos A, Ricou B, Suter PM, Morel DR, Morel P, et al. An improved method for isolation of microvascular endothelial cells from normal and inflamed human lung. *In Vitro Cell Dev Biol Anim.* 1998;34:529–536. doi: 10.1007/s11626-998-0112-z
73. King J, Hamil T, Creighton J, Wu S, Bhat P, McDonald F, Stevens T. Structural and functional characteristics of lung macro- and microvascular endothelial cell phenotypes. *Microvasc Res.* 2004;67:139–151. doi: 10.1016/j.mvr.2003.11.006
74. Gaskill C, Majka SM. A high-yield isolation and enrichment strategy for human lung microvascular endothelial cells. *Pulm Circ.* 2017;7:108–116. doi: 10.1177/2045893217702346
75. Mackay LS, Dodd S, Dougall IG, Tomlinson W, Lordan J, Fisher AJ, Corris PA. Isolation and characterisation of human pulmonary microvascular endothelial cells from patients with severe emphysema. *Respir Res.* 2013;14:23. doi: 10.1186/1465-9921-14-23
76. Wu S, Zhou C, King JAC, Stevens T. A unique pulmonary microvascular endothelial cell niche revealed by Weibel-Palade bodies and Griffonia simplicifolia. *Pulm Circ.* 2014;4:110–115. doi: 10.1086/674879
77. Humbert M, Guignabert C, Bonnet S, Dorfmüller P, Klinger JR, Nicolls MR, Olschewski AJ, Pullamsetti SS, Schermuly RT, Stenmark KR, et al. Pathology and pathobiology of pulmonary hypertension: state of the art and research perspectives. *Eur Respir J.* 2019;53:1801887. doi: 10.1183/13993003.01887-2018
78. Comhair SAA, Xu W, Mavrakis L, Aldred MA, Asosingh K, Erzurum SC. Human primary lung endothelial cells in culture. *Am J Respir Cell Mol Biol.* 2012;46:723–730. doi: 10.1165/rcmb.2011-0416TE
79. Wu X, Ma J, Ding D, Ma Y, Wei Y, Jing Z. Experimental animal models of pulmonary hypertension: development and challenges. *Animal Model Exp Med.* 2022;5:207–216. doi: 10.1002/ame2.12220
80. Cober ND, McCourt E, Godoy RS, Deng Y, Schlosser K, Qamsari ES, Azami J, Salehisiavashani E, Cook DP, Lemay S-E, et al. Mapping disease-specific vascular cell populations responsible for obliterative arterial remodeling during development of pulmonary arterial hypertension. *Cardiovasc Res.* 2025;cvaf146. doi: 10.1093/cvr/cvaf146
81. Singh N, Eickhoff C, Garcia-Agundez A, Bertone P, Paudel SS, Tambe DT, Litzky LA, Cox-Flaherty K, Klinger JR, Monaghan SF, et al. Transcriptional profiles of pulmonary artery endothelial cells in pulmonary hypertension. *Sci Rep.* 2023;13:22534. doi: 10.1038/s41598-023-48077-6

82. Zhou Y, Tabib T, Huang M, Yuan K, Kim Y, Morse C, Sembrat J, Valenzi E, Lafyatis R. Molecular changes implicate angiogenesis and arterial remodeling in systemic sclerosis–associated and idiopathic pulmonary hypertension. *Arterioscler Thromb Vasc Biol*. 2024;44:e210–e225. doi: 10.1161/ATVBAHA.123.320005
83. Godoy RS, Cober ND, Cook DP, McCourt E, Deng Y, Wang L, Schlosser K, Rowe K, Stewart DJ. Single-cell transcriptomic atlas of lung microvascular regeneration after targeted endothelial cell ablation. *eLife*. 2023;12:e80900. doi: 10.7554/eLife.80900
84. Caporarello N, Lee J, Pham TX, Jones DL, Guan J, Link PA, Meridew JA, Marden G, Yamashita T, Osborne CA, et al. Dysfunctional ERG signaling drives pulmonary vascular aging and persistent fibrosis. *Nat Commun*. 2022;13:4170. doi: 10.1038/s41467-022-31890-4
85. Niethamer TK, Levin LI, Morley MP, Babu A, Zhou S, Morrisey EE. Atf3 defines a population of pulmonary endothelial cells essential for lung regeneration. *eLife*. 2023;12:e83835. doi: 10.7554/eLife.83835
86. Wang G, Wen B, Guo M, Li E, Zhang Y, Whitsett JA, Kalin TV, Kalinichenko VV. Identification of endothelial and mesenchymal FOXF1 enhancers involved in alveolar capillary dysplasia. *Nat Commun*. 2024;15:5233. doi: 10.1038/s41467-024-49477-6
87. Zhang Q, Yaoita N, Tabuchi A, Liu S, Chen S-H, Li Q, Hegemann N, Li C, Rodor J, Timm S, et al. Endothelial heterogeneity in the response to autophagy drives small vessel muscularization in pulmonary hypertension. *Circulation*. 2024;150:466–487. doi: 10.1161/CIRCULATIONAHA.124.068726
88. Li J, Zhou Y, Shu T, Lei W, Tang Q, Yang Y, Zhang J, Chen W, Zhou B, Hu Q, et al. Differentiation of lung tissue-resident c-Kit+ cells into microvascular endothelial cells alleviates pulmonary vascular remodeling. *Dev Cell*. 2025;60:1601–1617.e7. doi: 10.1016/j.devcel.2025.01.010
89. Zhao G, Xue L, Weiner AI, Gong N, Adams-Tzivelekidis S, Wong J, Gentile ME, Nottingham AN, Basil MC, Lin SM, et al. TGF-βR2 signaling coordinates pulmonary vascular repair after viral injury in mice and human tissue. *Sci Transl Med*. 2024;16:eadg6229. doi: 10.1126/scitranslmed.adg6229
90. Zanini F, Che X, Knutsen C, Liu M, Suresh NE, Domingo-Gonzalez R, Dou SH, Zhang D, Pryhuber GS, Jones RC, et al. Developmental diversity and unique sensitivity to injury of lung endothelial subtypes during postnatal growth. *iScience*. 2023;26:106097. doi: 10.1016/j.isci.2023.106097
91. Ellis LV, Bywaters JD, Chen J. Endothelial deletion of p53 generates transitional endothelial cells and improves lung development during neonatal hyperoxia. *bioRxiv*. Preprint posted online May 8, 2024. doi: 10.1101/2024.05.07.593014
92. Guo M, Wikenheiser-Brokamp KA, Kitzmiller JA, Jiang C, Wang G, Wang A, Preissl S, Hou X, Buchanan J, Karolak JA, et al. Single cell multiomics identifies cells and genetic networks underlying alveolar capillary dysplasia. *Am J Respir Crit Care Med*. 2023;208:709–725. doi: 10.1164/rccm.202210-2015OC
93. Niethamer TK, Stabler CT, Leach JP, Zepp JA, Morley MP, Babu A, Zhou S, Morrisey EE. Defining the role of pulmonary endothelial cell heterogeneity in the response to acute lung injury. *eLife*. 2020;9:e53072. doi: 10.7554/eLife.53072
94. Fayyaz AU, Sabbah MS, Dasari S, Griffiths LG, DuBrock HM, Wang Y, Charlesworth MC, Borlaug BA, Jenkins SM, Edwards WD, et al. Histologic and proteomic remodeling of the pulmonary veins and arteries in a porcine model of chronic pulmonary venous hypertension. *Cardiovasc Res*. 2023;119:268–282. doi: 10.1093/cvr/cvac005
95. Raslan AA, Pham TX, Lee J, Kontodimas K, Tilston-Lunel A, Schmottlach J, Hong J, Dinc T, Bujor AM, Caporarello N, et al. Lung injury-induced activated endothelial cell states persist in aging-associated progressive fibrosis. *Nat Commun*. 2024;15:5449. doi: 10.1038/s41467-024-49545-x
96. Dorfmüller P, Günther S, Ghigna M-R, Thomas de Montpréville V, Boulate D, Paul J-F, Jaïs X, Decante B, Simonneau G, Dartevelle P, et al. Microvascular disease in chronic thromboembolic pulmonary hypertension: a role for pulmonary veins and systemic vasculature. *Eur Respir J*. 2014;44:1275–1288. doi: 10.1183/09031936.00169113
97. Kumar A, Raju S, Das A, Mehta AC. Vessels of the central airways. *Chest*. 2016;149:869–881. doi: 10.1016/j.chest.2015.12.003
98. Vincent M, Karolak JA, Deutsch G, Gambin T, Popek E, Isidor B, Szafranski P, Le Caignec C, Stankiewicz P. Clinical, histopathological, and molecular diagnostics in lethal lung developmental disorders. *Am J Respir Crit Care Med*. 2019;200:1093–1101. doi: 10.1164/rccm.201903-0495TR
99. Galambos C, Sims-Lucas S, Abman SH, Cool CD. Intrapulmonary bronchopulmonary anastomoses and plexiform lesions in idiopathic pulmonary arterial hypertension. *Am J Respir Crit Care Med*. 2016;193:574–576. doi: 10.1164/rccm.201507-1508LE
100. van der Have O, Westöö C, Ahrné F, Tian X, Ichimura K, Dreier T, Norvik C, Kumar ME, Spiekerkoetter E, Tran-Lundmark K. Shunt-type plexiform lesions identified in the Sugen5416/hypoxia rat model of pulmonary arterial hypertension using synchrotron-based phase-contrast micro-CT. *Eur Respir J*. 2022;59:2102802. doi: 10.1183/13993003.02802-2021
101. Westöö C, Norvik C, Peruzzi N, van der Have O, Lovric G, Jeremiasen I, Tran P-K, Mokso R, de Jesus Perez V, Brunnström H, et al. Distinct types of plexiform lesions identified by synchrotron-based phase-contrast micro-CT. *Am J Physiol Lung Cell Mol Physiol*. 2021;321:L17–L28. doi: 10.1152/ajplung.00432.2020
102. Kotloff RM, Thabut G. Lung transplantation. *Am J Respir Crit Care Med*. 2011;184:159–171. doi: 10.1164/rccm.201101-0134CI
103. Cui Y, Liu K, Monzon-Medina ME, Padera RF, Wang H, George G, Toprak D, Abdelnour E, D'Agostino E, Goldberg HJ, et al. Therapeutic lymphangiogenesis ameliorates established acute lung allograft rejection. *J Clin Invest*. 2015;125:4255–4268. doi: 10.1172/JCI79693
104. Li W, Gauthier JM, Tong AY, Terada Y, Higashikubo R, Frye CC, Harrison MS, Hashimoto K, Bery AI, Ritter JH, et al. Lymphatic drainage from bronchus-associated lymphoid tissue in tolerant lung allografts promotes peripheral tolerance. *J Clin Invest*. 2020;130:6718–6727. doi: 10.1172/JCI136057
105. El-Chemaly S, Malide D, Zudaire E, Ikeda Y, Weinberg BA, Pacheco-Rodriguez G, Rosas IO, Aparicio M, Ren P, MacDonald SD, et al. Abnormal lymphangiogenesis in idiopathic pulmonary fibrosis with insights into cellular and molecular mechanisms. *Proc Natl Acad Sci U S A*. 2009;106:3958–3963. doi: 10.1073/pnas.0813368106
106. Ebina M, Shibata N, Ohta H, Hisata S, Tamada T, Ono M, Okaya K, Kondo T, Nukiwa T. The disappearance of subpleural and interlobular lymphatics in idiopathic pulmonary fibrosis. *Lymphat Res Biol*. 2010;8:199–207. doi: 10.1089/lrb.2010.0008
107. Meinecke A-K, Nagy N, Lago GD, Kirmse S, Klose R, Schrödter K, Zimmermann A, Helfrich I, Rundqvist H, Theegarten D, et al. Aberrant mural cell recruitment to lymphatic vessels and impaired lymphatic drainage in a murine model of pulmonary fibrosis. *Blood*. 2012;119:5931–5942. doi: 10.1182/blood-2011-12-396895
108. Ge J, Shao H, Ding H, Huang Y, Wu X, Sun J, Que J. Single cell analysis of lung lymphatic endothelial cells and lymphatic responses during influenza infection. *J Respir Biol Transl Med*. 2024;1:10003–10003. doi: 10.35534/jrbtm.2024.10003
109. Humbert M, Sitbon O, Guignabert C, Savale L, Boucly A, Gallant-Dewavrin M, McLaughlin V, Hoeper MM, Weatherald J. Treatment of pulmonary arterial hypertension: recent progress and a look to the future. *Lancet Respir Med*. 2023;11:804–819. doi: 10.1016/S2213-2600(23)00264-3
110. Mirhadi E, Kesharwani P, Johnston TP, Sahebkar A. Nanomedicine-mediated therapeutic approaches for pulmonary arterial hypertension. *Drug Discov Today*. 2023;28:103599. doi: 10.1016/j.drudis.2023.103599
111. Boucetta H, Zhang L, Sosnik A, He W. Pulmonary arterial hypertension nanotherapeutics: new pharmacological targets and drug delivery strategies. *J Control Release*. 2024;365:236–258. doi: 10.1016/j.jconrel.2023.11.012
112. Deng Z, Kalin GT, Shi D, Kalinichenko VV. Nanoparticle delivery systems with cell-specific targeting for pulmonary diseases. *Am J Respir Cell Mol Biol*. 2021;64:292–307. doi: 10.1165/rcmb.2020-0306TR
113. Sun F, Wang G, Pradhan A, Xu K, Gomez-Arroyo J, Zhang Y, Kalin GT, Deng Z, Vagnozzi RJ, He H, et al. Nanoparticle delivery of STAT3 alleviates pulmonary hypertension in a mouse model of alveolar capillary dysplasia. *Circulation*. 2021;144:539–555. doi: 10.1161/CIRCULATIONAHA.121.053980
114. Liu B, Peng Y, Yi D, Machireddy N, Dong D, Ramirez K, Dai J, Vanderpool R, Zhu MM, Dai Z, et al. Endothelial PHD2 deficiency induces nitrative stress *via* suppression of caveolin-1 in pulmonary hypertension. *Eur Respir J*. 2022;60:2102643. doi: 10.1183/13993003.02643-2021
115. Yuan R, Liu M, Cheng Y, Yan F, Zhu X, Zhou S, Dong M. Biomimetic nanoparticle-mediated target delivery of hypoxia-responsive plasmid of angiotensin-converting enzyme 2 to reverse hypoxic pulmonary hypertension. *ACS Nano*. 2023;17:8204–8222. doi: 10.1021/acsnano.2c12190
116. Deng Z, Gao W, Kohram F, Li E, Kalin TV, Shi D, Kalinichenko VV. Fluorinated amphiphilic poly(β-amino ester) nanoparticle for highly efficient and specific delivery of nucleic acids to the Lung capillary endothelium. *Bioact Mater*. 2024;31:1–17. doi: 10.1016/j.bioactmat.2023.07.022
117. Gulati GS, D'Silva JP, Liu Y, Wang L, Newman AM. Profiling cell identity and tissue architecture with single-cell and spatial transcriptomics. *Nat Rev Mol Cell Biol*. 2025;26:11–31. doi: 10.1038/s41580-024-00768-2
118. Zormpas E, Queen R, Comber A, Cockell SJ. Mapping the transcriptome: Realizing the full potential of spatial data analysis. *Cell*. 2023;186:5677–5689. doi: 10.1016/j.cell.2023.11.003
119. Liu B, Yi D, Li S, Ramirez K, Xia X, Cao Y, Zhao H, Tripathi A, Qiu S, Kala M, et al. Single-cell and spatial transcriptomics identified fatty acid-binding

proteins controlling endothelial glycolytic and arterial programming in pulmonary hypertension. *Arterioscler Thromb Vasc Biol.* 2025;45:1145–1165. doi: 10.1161/ATVBAHA.124.321173

120. Tuder RM, Gandjeva A, Williams S, Kumar S, Kheyfets VO, Hatton-Jones KM, Starr JR, Yun J, Hong J, West NP, et al. Digital spatial profiling identifies distinct molecular signatures of vascular lesions in pulmonary arterial hypertension. *Am J Respir Crit Care Med.* 2024;210:329–342. doi: 10.1164/rccm.202307-1310OC
121. Zhao H, Ma X, Chen P, Liu B, Wei J, Zhang J, Desai AA, Frump AL, Rafikova O, Fallon MB, et al. Enhanced spatial transcriptomics analysis of mouse lung tissues reveals cell-specific gene expression changes associated with pulmonary hypertension. *J Respir Biol Transl Med.* 2025;2:10004. doi: 10.70322/jrbtm.2025.10004
122. Si Y, Lee JS, Jun G, Kang HM, Lee JH. Spatial omics enters the microscopic realm: opportunities and challenges. *Trends Genet.* 2025;41:774–787. doi: 10.1016/j.tig.2025.05.002
123. Alsafadi HN, Uhl FE, Pineda RH, Bailey KE, Rojas M, Wagner DE, Königshoff M. Applications and approaches for three-dimensional precision-cut lung slices. Disease modeling and drug discovery. *Am J Respir Cell Mol Biol.* 2020;62:681–691. doi: 10.1165/rcmb.2019-0276TR
124. Lehmann M, Krishnan R, Sucre J, Kulkarni HS, Pineda RH, Anderson C, Banovich NE, Behrsing HP, Dean CH, Haak A, et al. Precision-cut lung slices: emerging tools for preclinical and translational lung research: an official American Thoracic SOCIETY Workshop Report. *Am J Respir Cell Mol Biol.* 2025;72:16–31. doi: 10.1165/rcmb.2024-0479st
125. Stegmayr J, Wagner DE. The dawn of the omics era in human precision-cut lung slices. *Eur Respir J.* 2021;58:2100203. doi: 10.1183/13993003.00203-2021
126. Miao Y, Pek NM, Tan C, Jiang C, Yu Z, Iwasawa K, Shi M, Kechele DO, Sundaram N, Pastrana-Gomez V, et al. Co-development of mesoderm and endoderm enables organotypic vascularization in lung and gut organoids. *Cell.* 2025;188:4295–4313.e27. doi: 10.1016/j.cell.2025.05.041