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Pulmonary Circulation

Hot topics in the mechanisms of pulmonary arterial hypertension disease: cancer-like pathobiology, the role of the adventitia, systemic involvement, and right ventricular failure

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Abstract

In order to intervene appropriately and develop disease-modifying therapeutics for pulmonary arterial hypertension, it is crucial to understand the mechanisms of disease pathogenesis and progression. We herein discuss four topics of disease mechanisms that are currently highly debated, yet still unsolved, in the field of pulmonary arterial hypertension. Is pulmonary arterial hypertension a cancer-like disease? Does the adventitia play an important role in the initiation of pulmonary vascular remodeling? Is pulmonary arterial hypertension a systemic disease? Does capillary loss drive right ventricular failure? While pulmonary arterial hypertension does not replicate all features of cancer, anti-proliferative cancer therapeutics might still be beneficial in pulmonary arterial hypertension if monitored for safety and tolerability. It was recognized that the adventitia as a cell-rich compartment is important in the disease pathogenesis of pulmonary arterial hypertension and should be a therapeutic target, albeit the data are inconclusive as to whether the adventitia is involved in the initiation of neointima formation. There was agreement that systemic diseases can lead to pulmonary arterial hypertension and that pulmonary arterial hypertension can have systemic effects related to the advanced lung pathology, yet there was less agreement on whether idiopathic pulmonary arterial hypertension is a systemic disease per se. Despite acknowledging the limitations of exactly assessing vascular density in the right ventricle, it was recognized that the failing right ventricle may show inadequate vascular adaptation resulting in inadequate delivery of oxygen and other metabolites. Although the debate was not meant to result in a definite resolution of the specific arguments, it sparked ideas about how we might resolve the discrepancies by improving our disease modeling (rodent models, large-animal studies, studies of human cells, tissues, and organs) as well as standardization of the models. Novel experimental approaches, such as lineage tracing and better three-dimensional imaging of experimental as well as human lung and heart tissues, might unravel how different cells contribute to the disease pathology.

Keywords

experimental pulmonary hypertension, Pro-Con debate, right ventricle function and dysfunction, vascular remodeling

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Introduction

We summarize *hot topics* in pulmonary arterial hypertension (PAH) disease mechanisms, which were passionately debated in a public Pro-Con debate at the American Thoracic Society (ATS) International Conference 2018 in San Diego, CA, USA. Specifically, we will discuss the relevance and limitations of the cancer paradigm in PAH, the involvement of the adventitia in lung vascular remodeling, and the systemic nature of PAH, as well as the role of capillary rarefaction in right ventricular failure. Why were these topics chosen for a debate? In order to develop effective and disease-modifying therapeutics, it is crucial to understand the mechanisms of disease pathogenesis and progression to intervene appropriately. If we consider pulmonary vascular remodeling as a cancer-like disease, should we use cancer chemotherapeutics with their anti-proliferative properties as promising treatment approaches? Questions about the safety and tolerability of these drugs have raised many concerns regarding their use in PAH. Should we focus on improving endothelial dysfunction in PAH if we assume the ''inside-out'' hypothesis, that vascular remodeling starts with endothelial injury which sets of a cascade of events involving smooth muscle cell (SMC) proliferation and migration, inflammatory cell recruitment and activation of adventitial fibroblasts? Or should we rather focus predominantly on targeting the immune system and the adventitia as the initiating culprit of vascular remodeling, if the ''outside-in'' hypothesis is true. If PAH is a systemic disease, is it enough to focus on treating the pulmonary vascular bed, or should we rather develop more systemic approaches? Furthermore, can we use systemic manifestations of the disease as surrogate biomarkers for diagnosis and therapy? And last but not least, should we focus on the relative rarefaction of capillaries in the right ventricle (RV) in order to prevent heart failure, or would this be the wrong treatment target, as our current methodologies are not able to reliably assess the capillary density in the RV?

The following debate will shed some light on the above questions and show ways how to overcome some of the discrepancies.

Pulmonary arterial hypertension is a cancer-like disease

by Elena A. Goncharova

Key points:

- *Sustained proliferative signaling in pulmonary vascular cells*
- *Deficiency of tumor suppressor genes*
- *Resistance to apoptosis*
- *Deregulated cellular energetics*
- *Cancer-like replicative immortality—cell monoclonality and enhanced replicative potential*
- *Chronic inflammation and altered immune processes*
- *Genome instability, mutations, and DNA damage*

PAH is a progressive, fatal disease with high mortality rates and no cure.[1] Remodeling of small pulmonary arteries, a major and currently irreversible feature of PAH, is caused by increased proliferation and reduced apoptosis of resident pulmonary vascular cells.[2] It is becoming increasingly clear that PAH can be viewed and treated as a proliferative disease.[3,4] One of the most studied groups of proliferative diseases is human cancers, and multiple anti-cancer therapies are already available or in the pipeline. Caution, however, should be taken when repurposing cancer-focused drugs to treat hyperproliferation in PAH. Specifically, analysis of both the similarities and the differences between human cancers and PAH is needed to dissect shared molecular pathological components and develop new treatment options for PAH patients.

One unbiased way to determine the extent of similarities and identify cancer-shared mechanisms is to test the applicability of the classic Hanahan and Weinberg principles to the pathogenesis of PAH. Like PAH, human cancers have high heterogeneity, but there are several biological capabilities that are shared. Douglas Hanahan and Robert Weinberg proposed eight hallmarks of cancer: (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) resisting cell death, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) invasion and metastasis, (7) deregulating cellular energetics, and (8) avoiding immune destruction.[5–8] Underlying these hallmarks, there are two enabling characteristics, genome instability and inflammation, which support cancer development and growth by generating genetic diversity and foster several other hallmark functions.[8]

First proposed in 1998 by Rubin Tuder and colleagues,[9] the cancer theory of PAH has been further developed and expanded by many research groups. Below, I briefly highlight current findings supporting the cancer-like nature of PAH in the view of current hallmarks and enabling characteristics of cancer. For an in-depth review, I refer our readers to other studies.[3,7,10,11]

Sustaining proliferative signaling

Like cancer cells, pulmonary vascular cells from PAH lungs have increased proliferative potential and exhibit unstimulated growth in culture.[3,11] Growth signal autonomy is acquired by employing cancer-specific mechanisms, such as increased expression of mitogens coupled with activation of mitogen receptors;[12,13] constitutive up-regulation of key pro-proliferative/pro-oncogenic signaling pathways, such as mitogen-activated protein kinases, Akt, and mechanistic target of rapamycin;[14–18] and destruction of ''off'' switches/formation of self-supporting pro-proliferative signaling circuits (HIPPO-YAP/TAZ-ILK1, YAP/TAZ-miR-130/301).[19,20] Furthermore, similar to the growth-promoting interactions between cancer and stromal cells, there is evidence for heterotypic signaling between pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth

muscle cells (PASMCs),[21] and likely between macrophages and adventitial fibroblasts in PAH.[22]

Evading growth suppressors

Like human cancers, PASMCs in human PAH have inactivation or deficiency of key tumor suppressors, including PTEN, LATS1, FoxO1, p53, and Rb. This effect plays an important role in driving PASMC hyperproliferation, pulmonary vascular remodeling, and overall PAH.[19,23–28]

Resisting cell death

Resistance to apoptosis is another feature that is shared by cancer cells and pulmonary vascular cells in PAH. Resistance to apoptosis by microvascular PASMCs and PAECs in PAH is supported by the overexpression of anti-apoptotic proteins (Bcl-2, survivin, STAT3) and deficiency of pro-apoptotic proteins (Bax, Bim, p53).[9,14,29–31] Notably, protein-kinase Akt, which mitigates apoptosis in a substantial fraction of human tumors, is constitutively up-regulated in PASMCs in human PAH lungs and is required for the development of pulmonary hypertension (PH).[14,32]

Deregulating cellular energetics

Cancer-like metabolic reprograming is one of the key emerging hallmarks of PAH (reviewed in other studies[33–36]). Resident pulmonary vascular cells in PAH undergo a cancer-like metabolic shift to glycolysis (Warburg effect).[37–39] This is coupled with multiple cancer-like abnormalities in mitochondrial metabolism and dynamics in PAH PASMCs and the RV, including suppressed activity of mitochondrial pyruvate dehydrogenase and glucose oxidation, superoxide dismutase 2 deficiency, mitochondrial hyperpolarization and fragmentation, and dysregulated mitochondrial dynamics caused by down-regulation of the fusion protein mitofusin 2 and up-regulation of the fission protein DRP-1.[34,36,40] Elevated glutaminolysis and fatty acid oxidation have been reported as key metabolic abnormalities in PAH PAECs and PAH PASMCs,[41,42] further supporting similarities in metabolic rewiring between PAH and cancer.

Enabling replicative immortality

Both components of cancer-specific replicative immortality, cell monoclonality, and enhanced replicative potential are present in human PAH. Monoclonal proliferation has been reported in PAECs in PAH.[43] As in cancers, the protein component of telomerase that enhances replicative potential by preventing telomere shortening, Telomerase reverse transcriptase (TERT), is up-regulated in PASMCs from remodeled pulmonary arteries in idiopathic PAH (IPAH) lungs and supports pulmonary vascular remodeling and PH.[44]

Inflammation and immunity

As in human cancers, chronic inflammation and altered immune processes are important drivers of PAH pathogenesis.[45] Patients with PAH have elevated levels of circulating cytokines and chemokines, such as interleukin (IL)-1β, IL-6, IL-8, monocyte chemoattractant protein 1, fractalkine, CCL5/RANTES, and tumor necrosis factor α. Providing additional evidence of chronic inflammation and maladaptive immune response, there are increased perivascular inflammatory infiltrates (T- and B-lymphocytes, macrophages, dendritic cells, and mast cells) in PAH-remodeled pulmonary arteries, dysregulated Treg function, existence of PAH-specific remodeling-associated activated macrophages, and presence of autoantibodies against anti-nuclear antigens, endothelial cells (ECs), and fibroblasts. There is also an emerging role of PD1/PDL1 dysregulation in the pathogenesis of PAH, suggestive of cancer-like mechanisms to evade immune destruction.[33,45–49]

Genome instability and mutations

In addition to mutations of bone morphogenetic protein type 2 (*BMPR2*) and other members of the transforming growth factor β (TGF-β) family, which are strongly linked to a predisposition for PAH,[50] PAECs in PAH have genetic alterations associated with microsatellite instability and concomitant perturbation of expression of growth and apoptosis genes (*Smad9, TGFβRII, Rb1, BRCA2, Bax*), supporting monoclonal cell growth.[51] As in cancers, somatic chromosome deletions, increased mutagen sensitivity and DNA damage, and dysregulation of DNA repair-associated genes are also reported and play an important role in hyperproliferation of pulmonary vascular cells in PAH.[52–56]

In conclusion, there is an overwhelming number of similarities between PAH and cancer. Notably, 8 of 10 hallmarks and characteristics of cancer not only are present in PAH but also strongly impact disease pathogenesis. The existence of such fundamental similarities offers us an opportunity to employ certain cancer-specific strategies and repurpose already available anti-cancer drugs for the treatment of PAH. It should be noted however, that cell invasion and metastasis are not present in PAH, and the roles and regulatory mechanisms of angiogenesis differ between cancer and PAH. Thus, caution should be taken in repurposing cancer therapeutics for the treatment of PAH.

Pulmonary arterial hypertension is NOT a cancer-like disease

by Christophe Guignabert

Key points:

- *In contrast to cancer cells, pulmonary vascular cells from PAH patients exhibit a low proliferation rate and do not acquire the ability to proliferate uncontrollably*

- *These cells are sensitive to density-dependent inhibition of cell growth, and they generally differentiate normally and progress to their fully differentiated state*
- *These cells also maintain their cellular features and functions*
- *There is no constitutive activation of receptors (e.g. epidermal growth factor receptor) or known key regulators of the cell cycle*
- *Even if PAH occurs in a genetic context, the penetrance of mutations in genes predisposing to PAH is generally incomplete, and none of these mutations have been described in cancer*

Loss of growth control and loss of contact inhibition are two hallmark features of cancer cells, but not of pulmonary vascular cells in PAH. Currently, there is no evidence that pulmonary vascular cells, even in plexiform lesions, acquire the ability to reproduce uncontrollably in PAH. Furthermore, even if the balance between cell proliferation and cell death that normally maintains healthy tissue homeostasis is disturbed in PAH, both the rate of cell proliferation and the rate of cell death in PAECs, fibroblasts, and PASMCs from patients with IPAH are considerably closer to physiological conditions than are those in cancer cells.[37,57–62] It is also well documented that PAH pulmonary vascular cells have a limited lifespan in vitro, like any normal somatic cell. After a certain number of cell divisions, they enter senescence, which is morphologically characterized by enlarged, irregular cell shapes, and ultimately stop proliferating. In contrast to cancer cells, PAH pulmonary vascular cells also carry out their normal differentiation program and generally progress to a fully differentiated state.

The formation of efficient vascular networks, also known as tumor angiogenesis, is a critical hallmark in tumor development, but not in PAH. Even if high levels of various angiogenic factors, including fibroblast growth factor 2[59,60,63] and vascular endothelial growth factor (VEGF),[64] are present in the lungs of PAH patients, the angiogenesis process is clearly disordered or misguided, leading to a pattern of vascular rarefaction (dead-tree picture) that is characteristic of all forms of human PAH. This notion is further supported by the decreased capacity of PAECs from PAH patients to form vascular tubes in vitro, an observation that can be reproduced in ECs grown from induced pluripotent stem cells (iPSCs) derived from the skin of the same patient.[58,65] Since the total number of pericytes in pulmonary arterioles increases substantially during disease progression in human PAH[66] and defects in pericyte functions have been demonstrated,[67] a better knowledge of the contribution of circulating cells and resident vascular progenitors is needed.[66–68]

Cancer, but not PAH, is caused by mutations in oncogenes, tumor suppressor genes, and stability genes. Even when PAH occurs in a genetic context, the penetrance of mutations in genes predisposing to PAH is generally incomplete, and none of these mutations have been described in cancer.[69] For example, several types of cancer have a high incidence of *TP53* mutations, leading to the expression of mutant p53 proteins, but none of these mutations have been detected in PAH.

Although the pathogenesis of PAH is still incompletely understood, various stimuli, such as high glucose, insulin resistance, disturbed blood flow, and oxidative stress can partly explain metabolic reprograming or dysfunction of PAECs and their miscommunication with both resident vascular cells and immune cells in PAH.[70,71]

Therefore, it is clear that a tumorigenic mechanism alone cannot fully explain PAH. Yet, it should be conceded that the cancer-like concept has opened a new field of investigation regarding the potential use of anti-proliferative and/or oncologic drugs in PAH. However, questions about the safety and tolerability of these drugs raise many concerns regarding their use in PAH.[72,73]

The adventitia plays an important role in the initiation of pulmonary vascular remodeling

by Kurt Stenmark

Key points:

- *Changes in the adventitia in many vascular injury models precede those in other compartments and are in fact required for remodeling*
- *The highly complex adventitia consists of heterogeneous cells that release multiple factors upon injury, which can regulate differentiation, proliferation, apoptosis, migration, and collagen synthesis by other cells in the vessel wall*
- *There is evidence that adventitial fibroblasts can transform to myofibroblasts and migrate into the intima through the medial layer*
- *Inflammatory diseases of the vessel wall are largely orchestrated from the outside-in*
- *There is a relationship between adventitial angiogenesis (vasa vasorum) and the development of neointimal remodeling*
- *Resident stromal cells (fibroblasts) are involved in tertiary lymphoid organ (TLO) development in the lung*

The organizing committee of the Pulmonary Circulation Assembly at the ATS conference in 2018 was interested in examining the question of whether vascular remodeling in PH is initiated in the adventitia. Questions regarding initiation in human disease are obviously difficult, as the time of onset is usually not identifiable, at least not in most of the PH groups that have been identified to date. The question is, however, important, because it raises questions about the cells as well as the vessel layers (intima, media, and adventitia) that are potentially involved in the initiation, perpetuation, and persistence of vascular disease. It has been traditionally thought that the development of vascular lesions in both the systemic circulation and the pulmonary circulation follows an inside-out paradigm. This idea is

largely based on the observation that injuries to ECs often occur before other changes in the morphology and function of the vessel wall take place. It is well known that endothelial dysfunction causes activation of medial cells, often leading to neointimal formation as well as medial hypertrophy and fibrosis. Until recently, the adventitia has somehow been overlooked in this traditional concept.[74–76] In fact, the adventitia was ignored to the extent that many investigators stripped the adventitia from vascular specimens before the study. Yet emerging evidence leads us to recognize the possibility that the adventitia can serve as a staging ground for some of the earliest changes that occur in the vessel wall, especially inflammatory changes, and for medial and intimal changes. In the past 20 years, cumulative results have suggested that the adventitia is not merely a bystander in the pathogenesis of arterial disease, but rather, may represent a direct driving force for the development of vascular lesions.

Accumulating evidence, especially in the systemic circulation, has demonstrated that in many vascular injury models, changes in the adventitia precede those in other compartments and are in fact required for remodeling in response to various systemic vascular injuries.[77–81] This may be a consequence of the fact that the adventitia is a highly complex tissue and is poised to respond immediately to many of the stimuli involved in vascular remodeling. Unlike the intima and media, which are composed of single, although heterogeneous, types of cells, the adventitia contains, in addition to fibroblasts, leukocytes (including macrophages, dendritic cells, and mast cells), progenitor cells, nerves (sympathetic and parasympathetic), and lymphatics, as well as an additional blood supply, the vasa vasorum.[74,75] All of these cells have been shown to be collectively activated in response to injury and to release multiple factors that can regulate differentiation, proliferation, apoptosis, migration, and collagen synthesis by other cells in the vessel wall, including SMCs and ECs, through paracrine mechanisms. Clearly, many of these changes in cells have been ascribed to factors released by the injured endothelium. In support of adventitial involvement, many experimental models of vascular disease in the systemic circulation indicate early critical roles of the adventitia in vascular remodeling. These include experimental balloon/wire injury, hypercholesterolemia/atherosclerosis, transplant vasculopathies, and viral-induced vasculopathies.[81–87] In several studies in which blood vessels were injured from the intimal side (by wire or balloon), a rapid increase in cell proliferation was observed in the adventitia that exceeded that in the media at all time points after injury.[81,86,87] In these models, there was little accumulation of proliferating cells in the media and intima until at least seven days following an injury. In a large-animal model of hypercholesterolemia (porcine), it was shown that adventitial remodeling of the coronary artery, including adventitial angiogenesis, was an early change that preceded the development of changes in the intima and media.[83] Studies of human coronary artery specimens found that immature atherosclerotic plaques were surrounded by numerous adventitial macrophages, a finding supporting the idea that formation of the plaque is associated with early adventitial inflammation.[84] Further support for early adventitial involvement in vascular remodeling, even in the pathogenesis of intimal hyperplasia, was shown in experiments using LacZ (beta-galactosidase)-transfected adventitial fibroblasts. These cells were identified in the neointima following initial arterial injury.[88] These and other studies also showed that adventitial fibroblasts could transform into myofibroblasts and that these transdifferentiated cells could migrate into the intima through the medial layer. With regard to PH, at least in hypoxic rat models as well as in the neonatal calf model of PH, the earliest and most significant changes in proliferation occur in the adventitial compartment.[89,90] Whether these proliferative changes, though occurring earlier and more significantly than changes in the intimal and medial compartments, are a result of early endothelial injury remains unclear. Additional evidence for the important role of adventitial cells in early remodeling comes from studies showing that hypoxia-driven gene regulation in pulmonary artery fibroblasts results in a mitogenic stimulus for adjacent SMCs.[91]

There is strong evidence to suggest that inflammatory diseases of the vessel wall are largely orchestrated from the outside in.[74,77,80] It is increasingly appreciated that inflammatory responses are unique to the tissue where the inflammation occurs.[77,82] More recently, the idea has emerged that there is significant diversity in stromal cells, particularly in fibroblasts, and that function varies considerably among these subsets of cells, which have previously been lumped simply as "fibroblasts." Our recent data suggest that one subset of a fibroblast-like cell that exists in the pulmonary hypertensive vascular wall is characterized by inflammatory cytokine production that exceeds that of other fibroblasts, SMCs, and ECs.[62,92–94] Other fibroblast subsets exist that are functionally more similar to traditional myofibroblasts, while there are others that have anti-inflammatory properties. There is strong evidence that in the initial phases of PH in the animal models currently available, the earliest inflammatory responses occur in the adventitia.[92] In chronic persistent disease, this inflammation persists but then often involves both the medial and the intimal layers. This is consistent with the idea that in most normal arteries, the media is an immune-privileged site.[95] Human studies clearly demonstrate that the most intense inflammatory responses in late-stage human PH are observed in the adventitia.[96] Thus, we posit that, although the nature of initial damage to the vascular wall can vary with different types of injuries in both the systemic and the pulmonary circulation, mounting evidence strongly supports the idea that vascular inflammation may act as a driving force in the development of subsequent medial and intimal remodeling. Thus, it seems possible that inflammation represents a central mechanistic link between adventitial activation and vascular changes in response to a variety of stimuli.

It has been established that angiogenesis is important for wound-healing responses throughout the body. However, aberrant growth of new blood vessels is deleterious in pathologic conditions, which include diabetic retinopathy and tumorigenesis. Vascular injury is clearly associated with adventitial neovascularization in both the systemic and the pulmonary circulation.[74,97–99] A relationship between adventitial angiogenesis and the development of neointimal remodeling has been established in animal models of arterial injury in the systemic circulation.[100] We and others observed a tremendous expansion of the adventitial vasa vasorum in the pulmonary arteries of humans with PH and in large-animal models of PH. These vessels have been implicated in acting as conduits providing a pathway for further inflammatory cells to invade the vessel wall. More strikingly, recent information from two groups supports the idea that in the pulmonary circulation, this expansion of the vasa vasorum may result in establishing a connection with the venous circulation.[101,102] In fact, at the most recent World Congress, it was proposed that this may be a driving force in causing the venous changes that are now increasingly appreciated to occur in various forms of human PH.

Recent data suggest that the development of TLOs in PH is much more common than was previously thought.[103,104] These TLOs are thought to be the source of autoantibodies that can drive and promote the disease process. Intriguingly, there is strong experimental evidence to suggest that resident stromal cells, such as fibroblasts, in response to chronic inflammatory activation, as exists in various forms of PH and systemic vascular diseases, prime resident ECs to ultimately promote the growth of lymphatic vessels to aid antigen clearance and to foster the development of lymphoid-like tissue.[77] It has been shown that the presence of TLOs is associated with worse outcomes and morbidities. Because fibroblasts are critical for the development and maintenance of these organs, some researchers have gone so far as to propose that stromal cells should be targeted alongside the leukocyte component in TLO-associated pathologies.[77]

We must recognize that some of the conclusions we have drawn about the adventitia, both pro and con, are based largely on studies in mice and rats. Clearly, these species offer immense advantages for experimental work, as genetic and pharmacologic strategies for investigations of gain and loss of function have enabled enormous strides in understanding the functional consequences of various cell types and mediators. Recent studies using genetically engineered mice have also provided an opportunity to do lineage-tracing studies, which can provide important information on disease initiation and pathogenesis, at least in this species. However, it must be considered that these small-rodent studies are usually performed over a period of weeks, whereas diseases in humans, in particular, PH, develop over significantly longer periods of time. Furthermore, the functional attributes of cell populations and subpopulations in inbred mouse strains can often lead to more clear-cut answers than in studies of their human counterparts, where cellular and subcellular changes occur over a continuum rather than in black-and-white terms. As we move forward, we must integrate rodent studies with studies in larger animal models and continue to be driven by observations of human cells and tissues. It may be that pinpointing one cell as the originator and driver of PH is not within the realm of experimental probability.

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Vascular remodeling in pulmonary hypertension is NOT initiated in the adventitia

by Grazyna Kwapiszewska

Key points:

- *Only the remodeling of the intima and media is correlated with increased mean arterial pressure (mPAP) in PH patients*
- *Lineage-tracing experiments do not show a contribution of adventitial fibroblasts to the neointima*
- *Endothelial dysfunction as a result of EC-specific deletion of peroxisome proliferator-activated receptor gamma (PPARγ), BMPR2, or prolyl-hydrolase-2 (PHD2) causes spontaneous PH in mouse models and, by dysfunctional crosstalk, indirectly affects PASMCs*
- *SMCs expand during remodeling, and this expansion accounts for approximately 95% of remodeled vessels*
- *There is abundant evidence for the inside-out hypothesis of vascular remodeling*

Even though the adventitial layer contains inflammatory cell infiltrates, and their abundance correlates with the extent of vascular remodeling,[105,106] this does not imply causality because inflammatory cells are also present in all other layers of the pulmonary arteries.[96,107]

Vascular remodeling is characterized by narrowing of the small pulmonary arteries, which is mainly due to neointima formation (α-smooth muscle actin positivity) and some thickening of the media, but almost no changes in the thickness of the adventitial layer.[106,108,109] Furthermore, remodeling of the intima and media, but not of the adventitia, is correlated with mPAP.[106]

In murine models of vascular remodeling, lineage-tracing approaches have demonstrated that the number of platelet-derived growth factor receptor α (PDGFRα)-positive cells that determine fibroblasts does not expand and does not contribute to vascular remodeling.[110] Correspondingly, immunostaining in other PH models and lungs revealed that PDGFRα-positive cells are not detected in remodeled vessels (within α-smooth muscle actin-positive cells).[110]

If vascular remodeling is not initiated in the adventitia, which other cell types contribute to the remodeling of pulmonary arteries? Pioneering work has shown that thickened/edematous ECs, followed by endothelial apoptosis, are the first events in the vascular remodeling process.[111–113] Indeed, in recent years, it has been shown that endothelial dysfunction is the hallmark of vascular remodeling, and that crosstalk between ECs and SMCs is a key mechanism for PH development. In the hypoxic mouse model of PH, diverse growth and vasoactive factors secreted by the endothelium, such as endothelin-1 (ET-1) and Platelet Derived Growth Factor-subunit BB (PDGF-BB),[114,115] have a direct proliferative effect on neighboring PASMCs.

Most importantly, all current therapies are clinically effective in PAH target mediators affecting the EC/PASMC interaction or PASMC directly, namely ET-1, nitric oxide, and the prostacyclin pathway. Cumulatively, all lead to vasorelaxation of PASMCs.[116]

Direct evidence for the involvement of ECs in vascular remodeling and the development of PH has been demonstrated in transgenic animal models. Deletion of PPARγ or BMPR2 in ECs caused a spontaneous increase in right ventricular systolic pressure (RVSP), and the latter also increased inflammation.[117,118] Deficiency of PHD2 in ECs gives rise to obliterative vascular remodeling resembling that seen in IPAH patients.[119] PHD2 mice not only develop PH, but RVSP continues to increase over time. The underlying mechanisms include stabilization of hypoxia-inducible factor 2α (HIF2α) in ECs, decreases in caveolin 1 (CAV1), BMPR2, and apelin, and increases in ET-1 and IL-6, which cumulatively cause obliterative remodeling and severe PH.[119]

Deletion of HIF1α or PDGFRβ in ECs results in decreased SMC expansion and ameliorated PH.[120] In this study, the authors additionally provide the sequence of events leading to vascular remodeling in their PH model. In the initiation phase (days 1–3 of hypoxia), expression of HIF1α and PDGFRβ in ECs induces Krüppel Like Factor 4 (*KLF4*) expression in primary PASMCs and their migration. From day 5 to day 7, clonal expansion of PASMCs takes place, with final differentiation between days 14 and 21.[120]

What about PASMCs? Lineage tracing revealed that SMCs expand during remodeling and contribute to approximately 95% of remodeled vessels.[110,121] Deletion of crucial molecules modulating PASMC proliferation leads to protection from PH (Foxm1)[122] or increased RVSP (Foxo1, Hif1α, PPARγ).[23,123,124]

Cumulatively, previous and current reports point toward the inside-out hypothesis of vascular remodeling and rather minimal involvement of adventitia in the initiation and further development of pulmonary vascular remodeling. However, further studies with specific adventitial fibroblast drivers (which are currently lacking) could prove—or refute—the involvement of fibroblasts in the remodeling process.

Pulmonary arterial hypertension is a systemic disease

by Marlene Rabinovitch

Key points:

- *The presence of skeletal, coronary, and renal capillary abnormalities points to PAH as a systemic disease*
- *iPSC-derived ECs are abnormal in PAH*
- *Transplantation of bone marrow-derived cells from CAV1 knockout mice causes PH*
- *Systemic diseases (metabolic, infectious, and autoimmune) can cause PAH*

Recent work by a number of groups has provided evidence that PAH is a systemic disease. For example, both skeletal and coronary arterial abnormalities are associated with PAH. Recently, renal capillary abnormalities have been related to PAH. Our group has shown that iPSC-derived ECs and SMCs have abnormalities similar to those in native cells. Transplantation of bone marrow cells can recapitulate the full features of PH in a transgenic mouse with loss of CAV1. Finally, metabolic syndrome and infectious disorders such as schistosomiasis and HIV, as well as an autoimmune disease and Raynaud's phenomenon, are associated with PAH. These studies are described in more detail below.

In 2014, Potus et al.[125] showed that patients with PAH had rarefaction of skeletal blood vessels in association with impaired exercise tolerance independently of pulmonary vascular resistance. As early as 2011, Shimony et al. reported that the incidence of coronary artery disease was higher in PAH patients than in people of a similar age in the general population (28% vs 7.8%).[126] Meloche et al. in 2017 showed that coronary arteries from patients with PAH were more thick-walled than those of control subjects. The same result, i.e. almost a doubling of coronary arterial wall thickness, was evident in an experimental model of PH induced by the toxin monocrotaline.[127]

Nickel et al.[128] described renal dysfunction as a significant comorbidity in patients with PAH. At the ATS Scientific Sessions, Nickel showed a significant increase in the albumin:creatinine ratio, reflecting impaired renal function in patients with PAH associated with a *BMPR2* mutation when compared with unaffected mutation carriers and controls. This finding was further substantiated by extensive renal tubular damage and inflammation in mice with a homologous *BMPR2* mutation that developed PH. Most interesting was a recent report by Asosingh et al.[129] using *CAV1* transgenic mice that develop PH in hypoxia. Simply transplanting bone marrow from these mice was sufficient to induce PH.

Many systemic arterial diseases, such as scleroderma, are associated with PAH, as is digital ischemia or Raynaud's syndrome.[130] In addition, PAH is a major complication of systemic infections, such as schistosomiasis and HIV.[131]

Studies have described an extensive systemic bronchial arterial disease in PAH patients, most recently in 2016.[132] Most compelling are studies from our group by Sa et al.[65] showing that fibroblasts reprogramed to iPSCs and then differentiated to ''generic'' ECs have the same phenotype as native pulmonary arterial ECs, in that they have impaired angiogenesis, a result indicating that all ECs have dysfunction related to reduced BMPR2. Finally, PAH is associated with a high incidence of metabolic syndrome,[133,134] a systemic disease that complicates PAH, causing reduced survival and event-free survival.

Pulmonary arterial hypertension is NOT a systemic disease

by Norbert Voelkel

Key points:

- *Although PAH can be a manifestation of a systemic disease, it does not mean that it IS a systemic disease. IT IS NOT!*
- *In all associated forms of severe PAH, there is a requirement for the susceptibility of the lung vessels*
- *Sick lung vessels affect the heart and kidneys and are responsible for muscle microvasculopathy: the sick lung circulation is the cause of the systemically observed effects in PAH*
- *It is likely that resident vascular stem cells play a role in the evolution from lung vessel injury to ''wound healing gone awry''*

Very few things in PAH are categorically either black or white. Starting with the conclusion, one can safely state that severe PAH can be a lung organ manifestation of systemic disease. Examples are PAH associated with systemic sclerosis or sarcoidosis, POEMS syndrome, and tumor metastatic obliteration of small lung vessels.[135,136]

However, ''primary'' PAH—now called IPAH—is a disease that starts in the lung arterioles and progresses with the participation of bone marrow-derived cells, secondarily affecting the heart and skeletal muscle and leading to neuroendocrine activation. For short: the sick lung vessels affect the heart and the kidneys and are responsible for a muscle microvasculopathy.[125,137–139]

There are, as mentioned by Dr. Rabinovitch, several factors apparently supporting the view of a systemic disease cause or component of IPAH: circulating precursor cells, bone marrow-derived mast cells, dendritic cells, and megakaryocytes—which are found in and around the pulmonary vascular lesions, anti-vascular autoantibodies[140–143] and adipose tissue-derived inflammatory mediators,[144] and the association with the metabolic syndrome.[145] Yet, in all associated forms of severe PAH, there is a requirement for the susceptibility of the lung vessels—genetic or otherwise—that permits the vascular disease to take hold in the lung (the majority of patients with systemic sclerosis or sarcoidosis do not develop PH, and there are many *BMPR2* mutation carriers who never develop PH).

As in cancer pathobiology, one can apply the ''soil and seed'' concept. In this case, the prepared soil is the predisposed lung circulation that is being remodeled.

As an aside: the term ''pulmonary vascular remodeling'' and the recently coined ''de-remodeling'' are vague and not particularly helpful. Contractors and interior designers usually have a clear idea when they discuss the remodeling of a kitchen,[146] but apparently, each PH investigator has his or her idea about what constitutes and drives pulmonary vascular remodeling.[92,147,148]

To build a case for the lung-specific pathology that is the cause of severe PAH, we need only to consider that the cells that line the small lung vessels are lung-specific microvascular ECs,[149,150] that their maintenance is VEGF-dependent, and that lung EC somatic mutations can cause these ECs to proliferate and expand monoclonally.[43,151] Finally, the second hit that is required for severe angio-obliterative PAH to develop requires, as mentioned, some susceptibility that resides in the lung vessels.

One pathobiological concept of severe PAH, ''wound healing gone awry'',[152] paradoxically includes the loss of small lung vessels (the pruning of the vascular tree) due to the action of anti-angiogenic factors such as soluble fms-like tyrosine kinase, angiostatin, and others.[153,154] In this model, the disease is initiated by apoptosis of lung vascular ECs, leading to vessel loss. One example is the emphysema generated in wild-type rats after treatment with the VEGF receptor blocker, Sugen 5416.[155] Subsequent to or concomitant with the apoptotic loss of small vessels is—under the influence of a second hit—the evolution of an apoptosis-resistant EC phenotype that proliferates and somehow triggers an immune response packaged in and around the pulmonary arteriolar wall.[156]

This phenomenon is observed in the lung circulation only, and not in the heart or the skeletal muscles, which show vessel loss only (capillary rarefaction).[125,156]

An area that requires further investigation is the identification of lung vessel-specific resident stem cells. It is likely that they play a role in the evolution from lung vessel injury to ''wound healing gone awry.''[152]

To rephrase the ''systemic disease or not'' question, and conceding that extrapulmonary cells modulate the so-called pulmonary vascular remodeling,[152] we can ask whether there are any published data on models of severe PAH that illustrate the potential development of an autonomous lung vascular disease. Dai et al.,[119] in their remarkable study, demonstrated just that. They showed that knockout of *Egln 1*, encoding prolyl 4 hydroxylase 2 (to be sure: one single hit!) in the ECs of mice, was sufficient to increase the expression of the transcription factor HIF-2α in lung vascular ECs, with the consequence of spontaneously generating severe, angio-obliterative PAH. There were no other organ manifestations reported, and there was no systemic hypertension.

To return to the beginning: the pathobiology of the lung circulation, the "sick lung circulation," is a complex affair; extrapulmonary cells contribute to the vascular pathobiology, and in the opinion of this writer, the extrapulmonary manifestations observed in patients with IPAH[128,157] are not part of a systemic disease but consequences of the sick lung circulation. It remains unclear to what extent metabolic disorders[158] and comorbidities—including manifest diabetes[159]—contribute to the development of extrapulmonary vascular abnormalities in patients with severe PAH.

Capillary loss drives right ventricular failure in pulmonary arterial hypertension

by Harm J. Bogaard

Key points:

- *Compared with the well-adapted RV, there is reduced capillary density in the failing RV*
- *Capillary rarefaction has been attributed to impaired angiogenesis*
- *Targeting angiogenesis in the RV has improved RV function*
- *Two-dimensional histological quantification of the number of capillaries per cardiomyocyte, although imperfect, has been reproduced multiple times*
- *Stereological assessment of capillary numbers in RV failure documents a 50% increase in the cross-sectional area that a capillary needs to perfuse, likely resulting in cardiomyocyte malperfusion.*

Rarefaction is the loss of density of an item in a given volume or space. Rarefaction can result either from a reduction in the total number of items or from an increase in volume not matched by a proportional increase in the number of items. Most researchers would agree that in the setting of RV pressure overload, cardiomyocytes hypertrophy and capillaries attempt to keep up with the increase in tissue volume and oxygen demand through angiogenesis. We and others have described how the failure of RV adaptation to pressure overload may be due to insufficient angiogenesis, given the excessive degree of cardiomyocyte hypertrophy in PAH.[160,161] It has been demonstrated that capillary density remains normal in the well-adapted RV, whereas there seems to be an association between RV capillary rarefaction and deterioration in RV failure. The observations were based either on quantification of capillary volume per volume of RV tissue[160] or on the finding of a comparatively insufficient increase in the number of capillaries per cardiomyocyte, resulting in a reduced number of capillaries per tissue area.[161]

Mechanistically, capillary rarefaction has been attributed to impaired angiogenesis, either related to a loss in VEGF signaling[160,162] or due to reduced expression of the pro-angiogenic miRNA126.[161] However, the active disappearance of capillaries (e.g. due to endothelial apoptosis) was never fully excluded. Calculations suggesting capillary rarefaction in histological sections were corroborated by reduced molecular signals of angiogenesis and a metabolic shift toward nonaerobic energy generation.[163] Importantly, experimental interventions that resulted in a normalization of capillary density were shown to result in an improved function of the pressure-overloaded RV.[160,161,164]

Recently, meticulous quantification using stereological methods has suggested that the extent of capillary rarefaction in early studies was probably exaggerated.[165] This could very well be true, but it should not lead to a premature conclusion that capillary rarefaction is not present or is irrelevant in RV failure. First, it is unclear why conventional histological methods would always lead to a systematic error in the disadvantage of the failing RV. In other words, even if the methods of assessment are imperfect, does not the consistency of findings within and between studies suggest that capillary rarefaction is indeed present? Second, how can the discrepancy be explained between stereological findings and molecular analyses, which are clearly pointing in the direction of a loss in angiogenic signaling? Finally, stereology itself does seem to suggest that the oxygen supply to the tissues is indeed impaired in the failing RV. Graham et al. described an increase in the radius of tissue served per vessel from 14 to 17 μm.[165] This is equivalent to an increase in the cross-sectional area of perfused tissue from 616 to 900 m^2 and thus to a 50% increase in the area of tissue to be perfused by the same vessel. Given the fact that oxygen demand is massively increased in RV hypertrophy, this would imply severely impaired RV perfusion, particularly during exercise. Therefore, it seems that a failure in angiogenesis should still be viewed as critical in the transition from RV adaptation to failure.

Capillary loss does NOT drive right ventricular failure in pulmonary arterial hypertension

by Brian Graham

Key points:

- *Capillary rarefaction occurs in the RV in PH, in the setting of RV hypertrophy*
- *Capillary rarefaction is likely due to inadequate proliferation relative to the degree of hypertrophy, rather than vessel loss*
- *There are likely homeostatic mechanisms, as yet unclear, that work to maintain appropriate RV vascular density*
- *In severe or end-stage PH, there is likely suppression of homeostasis—through a decrease in pro-angiogenic factors and/or an increase in anti-angiogenic factors—which results in failure to maintain homeostasis of vascular density. The degree of RV vascular rarefaction is thus likely greater in more severe or end-stage PH*
- *RV capillary rarefaction results in an increase in the average perfusion radius per vessel. This rarefaction may*

critically limit the required delivery of O_2 and other metabolic substrates to RV tissue in exercise

- *Planimetry likely exaggerates the degree of rarefaction observed compared with stereology*

RV failure is the major cause of death in PH, but the determinants of RV failure are not well understood. More than 10 studies have reported that there is a decrease in the density of capillaries in the RV tissue of animals and humans with PH. I agree with these findings. However, I disagree with the interpretation that rarefaction is evidence of capillary loss.

An alternative interpretation of RV vascular rarefaction in PH is that there is significant hypertrophy of RV cardiomyocytes (which is well known) with a relatively inadequate proliferation of the vascular network to compensate for this hypertrophy. It is impossible to distinguish between vessel loss and relative underproliferation in the setting of hypertrophy by simply looking at histologic images (i.e. planimetry). Distinguishing between these two possibilities requires stereology: a set of methods to minimize bias in the analysis of tissue characteristics, which includes measurement of the reference volume.

Using stereology, our group has analyzed the vasculature of the RV free wall in specimens from humans, rats, and mice, with and without PH.[165,166] Across these specimens, we have observed that there is, in fact, a substantial increase in both RV volume and absolute length of the RV vasculature. In human and rat specimens, the magnitude of the increase in RV vascular length is slightly (but statistically significantly) less than the magnitude of the increase in RV volume. This ratio of the change in tissue length to the change in tissue volume results in a modest but real decrease in the density of the vasculature; in the mouse specimens, we found no change in RV vascular density. Furthermore, in the rat and mouse specimens, we observed many proliferating but no apoptotic ECs. Analysis of mouse RV tissue using stereology has also been reported by Kolb et al. with similar results.[167]

We also performed metabolomic analysis of the rat and mouse RV specimens and found significant metabolic differences in the diseased RVs. However, there were no decreases in the concentrations of glucose, glutamine, or hydroxybutyrate, and we found no evidence of hypoxia, at least at the steady state. It is quite possible that with exercise, the RV vascular rarefaction results in inadequate substrate delivery at a lower threshold; this remains to be tested.

In summary, we find evidence that there is significant proliferation, not the loss, of the vasculature in the RV in PH. We find that the degree of RV vascular proliferation in humans and rats with PH is less than the degree of RV hypertrophy, resulting in modest vascular rarefaction. I interpret these data as evidence of homeostatic mechanisms that promote the maintenance of appropriate RV vascular density. In the failing RV, there may be an interruption of these homeostatic mechanisms that result in inadequate vascular adaptation through down-regulation of homeostatic drivers and/or up-regulation of anti-angiogenic factors. A consequence of the resulting rarefaction may be inadequate delivery of oxygen and other metabolites during exercise, which may contribute to RV failure.

Conclusion

Although the debate was not meant to result in a definite resolution of the pro and con sides of the specific arguments, it sparked ideas about how we might resolve the discrepancies. Improving our disease modeling by integration of rodent models of the disease with large-animal studies and studies of human cells, tissues, and organs, as well as standardization of the models, would allow for a better understanding of the pathogenesis of the disease that is relevant to the human disease and could potentially result in the development of more effective therapies. Novel experimental approaches, such as lineage tracing and better three-dimensional imaging of experimental as well as human lung and heart tissues, might unravel how different cells contribute to the disease pathology and clarify the ambiguous terminology of capillary rarefaction versus vessel loss versus vascular remodeling.

Conflict of interest

ES has served as a scientific adviser for Selten Pharma Inc, Vivus (Modest). WMK has served as a consultant for Boehringer Ingelheim Pharma GmbH & Co KG (Modest) and GlaxoSmithKline Research & Development (Modest). HJB has received research grants from GSK, Actelion, and Therabel. The other authors have no other conflicts of interest to disclose relevant to this article.

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