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Tensions in Taxonomies: Current Understanding and Future Directions in the Pathobiologic Basis and Treatment of Group 1 and Group 3 Pulmonary Hypertension

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Abstract

In the over 100 years since the recognition of pulmonary hypertension (PH), immense progress and significant achievements have been made with regard to understanding the pathophysiology of the disease and its treatment. These advances have been mostly in idiopathic pulmonary arterial hypertension (IPAH), which was classified as Group 1 Pulmonary Hypertension (PH) at the Second World Symposia on PH in 1998. However, the pathobiology of PH due to chronic lung disease, classified as Group 3 PH, remains poorly understood and its treatments thus remain limited. We review the history of the classification of the five groups of PH and aim to provide a state-of-the-art review of the understanding of the pathogenesis of Group 1 PH and Group 3 PH including insights gained from novel high-throughput omics technologies that have revealed heterogeneities within these categories as well as similarities between them. Leveraging the substantial gains made in understanding the genomics, epigenomics, proteomics, and metabolomics of PAH to understand the full spectrum of the complex, heterogeneous disease of PH is needed. Multimodal omics data as well as supervised and unbiased machine learning approaches after careful consideration of the powerful advantages as well as of the limitations and pitfalls of these technologies could lead to earlier diagnosis, more precise risk stratification, better predictions of disease response, new sub-phenotype groupings within types of PH, and identification of shared pathways between PAH and other types of PH that could lead to new treatment targets.

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Introduction

In the over 100 years since the recognition of pulmonary hypertension (PH), immense progress and significant achievements have been made with regard to understanding the pathophysiology of the disease (22, 234). These advances have been mostly in idiopathic pulmonary arterial hypertension (IPAH), which for almost 100 years was known as primary pulmonary hypertension (PPH) and was the paradigmatic disease in the field of PH. Beginning with the first World Health Organization (WHO) Symposium on PH in Geneva in 1973, clinicians and researchers attempted to define PH using hemodynamic parameters while delineating distinct categories of PH according to histopathology and presumed mechanism, at that time describing three patterns: plexiform pulmonary arteriopathy, pulmonary veno-occlusive disease, and chronic thromboembolic disease (71). Twenty years later at the Second World Symposia on PH, a 5-group classification of PH was established (Table 1) (58). Pulmonary arterial hypertension (PAH), termed Group 1 PH, was defined as a resting mean pulmonary artery pressure (mPAP) greater than or equal to 25 mmHg, a pulmonary capillary wedge pressure (PCWP) less than or equal to 15 mmHg, and a pulmonary vascular resistance (PVR) greater than or equal to 3 Wood units, and was characterized histopathologically by concentric laminar intimal proliferation of medium and small sized pulmonary arteries and plexiform lesions; Group 2 encompassed patients with PH due to left heart disease; Group 3 comprised PH associated with respiratory disorders or hypoxemia, Group 4 was defined as chronic thromboembolic pulmonary hypertension (CTEPH), and Group 5 PH included other diseases known to alter the pulmonary vasculature and promote PH (167). Since then, four additional World Symposia have been held, most recently in Nice in 2018, each time incorporating advances in the understanding of the clinical presentation, pathophysiology, and pathology of PH including the identification of genetic and familial causes, adding to an ever-growing list of diseases categorized as Group 1 PH.

However, there is a growing recognition of the heterogeneity of PAH (176, 204), likely paralleling the significant molecular and clinical heterogeneity of all groups of PH, especially in what is currently termed Group 3 PH. While the modification of the PH classification system is largely driven by the study of IPAH, it is unclear whether the insights gained in IPAH can be extended to other forms of PH, which are more frequent and have a larger impact on morbidity and mortality than IPAH. Shortcomings of the PH classification are further pronounced considering the discrepancies within and limited understanding of the varied entities including hypoxia, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis all clustered within Group 3 PH, implying a common nature to these disparate entities.

While the development of several pulmonary vasodilator therapies has dramatically improved the quality of life and survival of PAH patients, the mainstay of treatment of Group 3 PH has largely remained oxygen delivery and addressing the underlying cause of hypoxemia. Only one drug, inhaled treprostinil, has been approved by the Food and Drug Administration (FDA) for the treatment of PH due to interstitial lung disease (ILD), and this was derived from existing inhaled vasodilator therapy for PAH (220). There is a pressing unmet need for advances in treatment options for PH in lung disease, where prevalence is

high and outcomes are poor, affecting 38% to 80% of idiopathic pulmonary fibrosis (IPF) patients (106, 126, 166) and 25% of patients with mild to moderate COPD, but up to 90% in those with severe Global Initiative for Chronic Obstructive Lung Disease (GOLD) Stage IV COPD (18, 87). PH carries a markedly higher mortality in patients with chronic lung disease compared to those without PH. Alarmingly, some epidemiologic studies have found that Group 3 PH has equally poor if not worse mortality compared to PAH patients despite a lower severity of PH and right ventricle (RV) dysfunction (Figure 1) (50, 138), highlighting the urgent need to understand the pathobiologic underpinnings of group 3 PH and to discover new therapies.

The mechanisms contributing to the development of PH in lung diseases remain poorly understood. Hypoxic-mediated vasoconstriction has long been thought to be the primary driver of all forms of group 3 PH; however, PH can develop in both COPD and IPF in the absence of hypoxemia, and correction of hypoxemia with supplemental oxygen therapy only partially reverses PH (9, 31, 49). Other key pathobiological concepts that have been promoted include PH due to the destructive lung process itself and the resultant excessive vasoconstriction and vascular destruction leading to vessel paucity, excessive vascular growth, and inflammation.

The complexity of these paradigms and their potential interactions underlines the difficulty in finding a unifying or prevailing hypothesis in PH associated with chronic lung diseases. The limited understanding of the pathobiology of Group 3 PH may have been the basis of the taxonomy developed in the initial World Symposia, but in this new era of advanced technologies including single-cell sequencing and molecular phenotyping, this classification requires re-examination aimed at revealing how similar these conditions are to one another or to Group 1 PH. Despite the significant impact, inroads, and developments resulting from the recognition of PH as an entity and its initial classification scheme, the continued grouping of dissimilar pathobiologic processes under one umbrella may now impede the ability to study and recognize heterogeneous mechanisms implicated in not only Group 3, but also Group 1 PH, and thus contribute to the paucity of novel therapies for these diseases.

Advances in the genetic characterization of the disease as well as the advent of “omic” technologies have revealed extensive heterogeneity among the Group 1 PH diseases. Machine learning (ML) models have been applied to understand the pathobiology of PAH and have revealed heterogeneities across entities that were once considered to be etiologically homogeneous (86). These findings are consistent with clinical observations that some patients respond to certain pharmacologic therapies, while others do not (118). These approaches have formed the basis for interpreting other forms of PH in the framework of Group 1 PH, but the dangers related to inappropriate translation of these findings to highly complex diseases, such as COPD and pulmonary fibrosis, must be acknowledged.

Our goal is to provide a state-of-the-art review of the understanding of the pathogenesis of Group 1 PH and Group 3 PH, and in so doing will highlight significant knowledge gaps in the underlying molecular pathology of these highly prevalent forms of PH. We will also emphasize new scientific advances in the era of pulmonary vascular multimodal “omics,” using comprehensive high-throughput molecular analyses that can enable more precise

phenotyping within the current umbrellas of PH. Given the multidimensionality of new data that can be generated in the present age of molecular medicine, continued adherence to the compartmentalized knowledge of these insights does not capture how best to understand PH across its disease spectrum. These approaches can aid in uncovering previously hidden similarities and key differences in diseases currently residing under different classifications (128). In the future, machine learning and artificial intelligence may provide one path to the discovery of novel, personalized therapies in all forms of PH.

The Normal Pulmonary Vasculature

The healthy pulmonary circulation is a low-pressure, high-compliance system, allowing the RV to accommodate high blood flow (i.e., cardiac output, Q) with minimal work and preventing fluid from escaping the pulmonary vessels into the lung tissue. PVR is related to the pressure and flow through the pulmonary circulation, summarized by the equation PVR = (mPAP – PCWP)/Q. A large meta-analysis performed by Kovacs et al. demonstrated that in healthy individuals resting mPAP typically falls below 20 mmHg (mean 14.0 ± 3.3 mmHg); in the same review, PVR in healthy individuals was 0.93 ± 0.38 WU (97). PVR may also be described by Poiseuille's law, where PVR is proportional to the product of the volume flow rate, viscosity coefficient, and tube length, and inversely proportional to the tube radius to the fourth power (123). Increased volume flow can be accommodated through pulmonary vascular recruitment and/or distensibility (98). Thus, reduction in pulmonary arteriolar vessel caliber (the radius in Pouiselle's equation) due to increased vascular tone or remodeling can markedly increase the resistance across the pulmonary vasculature. As will be described in this article, hypoxic pulmonary vasoconstriction and pulmonary vessel wall remodeling in the various diseases of PH increase PVR and thus increase pulmonary arterial pressure.

Pathobiology of Group 1 PH

The age of vasoconstriction

As early as in the 1950s, clinicians and investigators defined PAH using physiologic and hemodynamic descriptions and focused their efforts on understanding its pathogenic mechanisms. A period that could be termed "the age of vasoconstriction" then followed until the early 1990s, during which much work was focused on understanding the functional mechanisms governing pulmonary vascular tone that would drive vasoconstriction and the ensuing pulmonary pathological lesions of PAH. The vasoconstriction paradigm was centered upon the identification of hypoxic vasoconstriction, which led to the discovery of chemical mediators of vasoconstriction or vasodilation, and from these, the development of therapeutic agents that could counter excessive vasoconstriction or promote vasodilation: prostacyclin analogs (219), endothelin receptor blockers (155), and pharmacologic enhancers of endogenously produced nitric oxide (159), all of which remain the mainstays of the modern PAH treatment (80).

A focus on pulmonary vascular remodeling

By the late 1980s, it became increasingly clear that vasoconstriction alone could not completely explain PH pathophysiology, as in many patients there was a “fixed” component of PH due to pulmonary vascular remodeling that was unresponsive to vasodilator therapy (149, 170). Accordingly, the field shifted its focus to study the mechanisms of pulmonary vascular remodeling, initially by identifying the cell types contributing to medial and adventitial thickening, as these were the changes most evident in the hypoxic animal models of the disease which were dominant at the time (117, 180). An overabundance of adventitial fibroblasts and medial smooth muscle cells (SMCs) was consistently observed in these compartments (180). These studies also pointed out that SMCs and adventitial fibroblasts, rather than behaving uniformly, are each phenotypically heterogeneous populations with diverse functions responding in distinct ways to specific stress stimuli (39, 56, 225). However, animal models of chronic PH due to environmental hypoxia (209), or for that matter monocrotaline injection (141), fall short of fully capturing the type and extent of pulmonary vascular remodeling seen in severe forms of human PAH (179, 212). The lungs of most patients with IPAH, the prototypic form of severe PH in humans, demonstrate a complex variety of vascular lesions which cannot be reproduced by chronic environmental hypoxia or monocrotaline. While the newer model of PH involving administration of the vascular endothelial growth factor (VEGF) inhibitor Sugen followed by hypoxic exposure may perhaps model human PAH more closely, it likewise does not recapitulate the cellular complexity of the human condition (2, 188, 192). Further, the relatively short time frame of pulmonary vascular remodeling in hypoxic rodent models (i.e., up to 5–7 weeks, and usually imposed on young, growing animals) is unlikely to capture the diverse and dynamic temporal changes present in the human disease, which at presentation comprises a large age span and has widespread involvement of the pulmonary vascular bed.

The cancer hypothesis of pulmonary hypertension

In the 2000s, newly available technologies opened up possibilities of understanding the molecular, metabolic, and genetic/epigenetic basis of the disease (32, 42, 229), leading to discoveries that framed a new concept, the so-called “cancer-like hypothesis of PH.” This hypothesis, largely based on human studies, addressed mechanistically the pathogenesis of complex vascular lesions in PAH (211). Plexiform lesions in human IPAH lungs were found to be monoclonal, which strongly suggested that stem- or progenitor-like cells could acquire mutations enabling either increased cell proliferation or decreased cell death (102, 202), becoming genetically unstable (5, 51, 230), losing suppressive cell growth signals (such as the transforming growth factor-β family), and activating signaling pathways to favor their long term survival (114). The cancer-like paradigm flourished and enabled the identification of ever more complex signaling pathways involved in cellular metabolism, extracellular matrix (ECM) remodeling, and alterations of the immune system, among others (66, 140, 147, 173, 191). Importantly, work in this area led to many potentially exciting new ideas regarding therapeutic targets (Table 2) (140).

However, as occurs with any novel disease hypothesis, contradictions and controversies have arisen, including the recognition that pulmonary vascular cells behave differently than cancer-like cells, including their low proliferation rate, sensitivity to density-dependent

inhibition of cell growth, and conservation of cellular features and functions rather than de-differentiation, raising questions about the safety and efficacy of using anti-neoplastic drugs in the context of PAH (Figure 2) (66, 173). Without a critical reassessment and proper understanding of the origins, strengths, and limitations of the cancer hypothesis, there is a risk that early closure and acceptance of this as a "unifying hypothesis" for PAH can limit further advancements in the field through the exploration of new and different paradigms (34, 204).

Initiation of PAH

A significant unresolved issue in human Group 1 PH is lack of understanding of the factors involved in initiation of disease. The nature of the triggering event is unclear but has been speculated to be due to endothelial injury or death potentially due to an undefined infectious agent. This is supported by the incomplete penetrance of gene mutations found in heritable PAH, most notably bone morphogenetic protein II (BMPR2), suggesting a triggering event is needed in addition to a genetic predisposition (46, 82). It has been inferred that this hypothesis is correct based on animal studies using in particular the Sugen/hypoxia rat model, wherein a transient induction of endothelial cell (EC) death of apoptosis leads to development of complex vascular lesions (188). However, this is as of yet an unproven assumption in human PAH.

Profiling of pulmonary vascular lesions

By the time of diagnosis, patients with PAH show established, potentially irreversible, pulmonary vascular lesions. At present, there is no causal link or association between a particular type of lesion and the marked increase in PVR seen in PAH (83, 194). Several lesions are recognized either topographically as located in the intima, media, adventitia, or by the predominance of specific structural cells that compose each of the specific lesions (200): ECs likely predominate in plexiform and some concentric intima lesions (199), myofibroblasts are predominant in intima obliteration, SMCs account for media remodeling, and fibroblasts undergo activation in the adventitia. Few mapping studies have localized some obliterative lesions to branching points (35, 227), or supernumerary arteries (195), but how vascular lesions are distributed along the fractal pulmonary artery remains unclear (195). These changes of resident pulmonary vascular cells often occur in the context of perivascular inflammation, one of the most consistently reported findings in human and animal models of PH (Figure 3); importantly, perivascular inflammation is more than a bystander in the disease process, as it impacts on hemodynamic parameters (70, 160, 176, 199). None of the individual intimal or medial lesions carry a statistically significant correlation with pulmonary artery pressures or PVR (176). However, a trend is noted when hemodynamics are compared with "intima thickening." Furthermore, the quantification of each type of lesion varies significantly in different lung regions. There is no correlation among lesions in their distribution or frequency, limiting the extent to which these data might offer clues to their potential temporal, topographical and pathogenetic relationship (176). The aspect of lesional heterogeneity is illustrated by the inability to distinguish clearly diseased lung clusters as assessed by principal component analysis based on data related to intima or medial remodeling, the number of profiles of plexiform lesions, and the degree of perivascular inflammation (176). It is also important to note that each of these

elements of diversity and potentially plasticity require detailed consideration not only in PH but also in the normal pulmonary circulation. Importantly, age-related phenotypic changes in the apparently normal pulmonary circulation might differ significantly across the lifespan (176). As detailed below, the introduction of molecular profiling of these specific vascular lesions will likely offer unique insights into their molecular characteristics and the degrees that PAH lungs differ among patients with the disease.

The extracellular matrix in PAH

Another highly important participant in the vascular lesions in PAH is the ECM. The ECM, which is largely understudied in the PH field, is a determinant of location- and time-dependent plasticity of pulmonary vascular cells. The ECM remains in constant flux as the lung matures, ages, and responds to injury (191). ECM remodeling and pulmonary vascular stiffness occur early in the disease process and often precede increases in vascular thickness and even arterial pressure. This suggests changes in ECM and related proteins can be causal and not simply the result of changes in vascular cell phenotypes. In established IPAH, there are distinct, compartment-specific changes in the expression of genes encoding collagens, tenascin, fibronectin, and osteopontin. Additionally, there is evidence of the ongoing breakdown of elastin and collagens with increased expression of elastases and metalloproteinases (75, 78, 140). ECM remodeling and stiffness, through mechano-activation of multiple signaling pathways, can have profound effects on vascular cell phenotype and function (4, 43, 44, 190, 216). The fine proteome of pulmonary vascular lesions in human PH (notably in PAH) has not been elucidated thus far. We propose that the intra- and extracellular proteome are key to the thickening of all three vascular compartments and that there are multiple stages of matrix remodeling defined by unique macromolecular components, matrix cross-linking, and cellular signatures that need to be defined. The proteome of specific vascular lesions is predicted to contain disease driver(s), such as elements of the classic and alternative pathways of complement (57). Moreover, this lesional proteome may ultimately manifest increased pulmonary vascular stiffness and drive pathogenic enhanced cell survival/proliferation, resistance to apoptosis, and inflammation (78).

Recognizing heterogeneity within PAH

Biology and medicine have recently experienced rapid growth in groundbreaking methodologies that can be applied to interrogate fundamental cell and molecular processes. These can be used to resolve the mechanistic hierarchy of specific pathways concerning disease initiation and progression, taking into consideration the critical nature of a highly heterogeneous disease. This heterogeneity exists in clinical initiating factors, such as age at onset, clinical presentation, rate of progression, and response to therapy (184). Importantly, between- and even within-patient heterogeneity involving the types of pulmonary vascular lesions and the corresponding endotypes (i.e., underlying molecular processes driving the specific disease presentation) have been uncovered by our and other groups and underlie the almost certain high complexity of disease pathogenesis (76, 84, 94, 176). We believe that the paucity of investigations into defining these aspects of heterogeneity has resulted in a lack of recognition of subphenotypes of PAH and thereby an understanding of specific factors contributing them, hindering the development of more individualized, targeted therapies.

Fortunately, there are emerging technologies that will allow us to begin to address the important issues defined above that we will address later in this article.

Pathobiology of Group 3 PH

Epidemiology of Group 3 PH

PH is a common complication of many lung diseases. The prevalence of PH associated with IPF-PH is estimated to range from 38% to 80% of patients (106, 126, 166). When compared to IPF without PH, IPF-PH patients have worse clinical outcomes, including decreased functional status, increased oxygen requirements, and, most importantly, a 5- to 8-fold increase in 1-year mortality (93, 106, 120). A significant proportion of individuals with COPD also develop PH, with the prevalence ranging from 30% to 70% (27, 88, 119, 131). Despite being associated with only mild to moderately elevated pulmonary artery pressure, PH in COPD is a major cause of morbidity and mortality in COPD, with an increased risk of exacerbations and decreased survival (28, 88, 119, 131). High altitude PH, first described in the 1950s, is observed in individuals chronically residing at altitude >2500 m (approximately 8000 ft) (103), putting approximately 140 million people around the world at risk for a disease with reported prevalence of <1% to 18% (20, 21, 61, 108, 127, 172).

Despite the recognition of the need for treatment in Group 3 PH, it remains unknown whether treatment with the pulmonary vasodilator therapies used in Group 1 PH is appropriate. Most published data using approved PAH therapies demonstrated that they are ineffective in Group 3 PH, and in some cases have shown increased mortality (36, 81, 95, 125, 145, 146). The oral and systemic pulmonary vasodilators which are used to treat PAH can worsen ventilation/perfusion mismatch in COPD (28, 119). To date, seven randomized control trials have been conducted for COPD-PH, with relatively low number of subjects, but without consistent outcomes to consistently support the use of pulmonary vasodilator use (17, 62, 148, 182, 206, 210, 213). Results from the recently completed INCREASE trial suggest benefit from inhaled treprostinil (prostacyclin) in patients with PH due to ILD (205). Still, the development of novel medical therapies for Group 3 PH is an urgent unmet need (125) but is currently limited by our poor understanding of its pathogenesis.

Comparing pathologic findings of Group 1 and Group 3 PH

Barriers to progress in understanding and finding treatments in Group 3 PH include a relative paucity of studies on the pathology of Group 3 PH compared to Group 1 PH and to differences in methodologies that make direct comparisons between these states difficult.

In studies of chronic high-altitude PH, a disease thought largely to be characterized by pure hypoxia-mediated PH, autopsies of long-term high-altitude dwellers reveal hypertrophy of the media of proximal pulmonary arteries and increased muscularization of distal arterioles with an increased ratio of distal to proximal arteries (215). Similarly, patients with COPD-PH develop hypertrophy of the muscular tunica media layer (113, 226) while the smaller arterioles develop a smooth muscle layer with a new internal elastic lamina (223). Pathology of COPD-PH has also been characterized by hyperproliferation of the intima and a thickened

tunica media, both of which are primarily composed of SMCs (157, 200). COPD patients undergoing lung transplantation with severe PH had an increased remodeling score of the microvessels compared to COPD patients with moderate PH or without PH (23), which is not typical of remodeling in PAH. Animals exposed to cigarette smoke develop changes in the intimal and medial layer of small pulmonary arteries which are similar to those seen in humans (64, 113, 162). In IPF-PH, vascular remodeling is also seen prominently in the intimal and medial layers (76). In a study which compared well-characterized lungs explanted from COPD-PH and IPF-PH patients, the smaller vessels in COPD-PH lungs (<200 μm) exhibited more pronounced lumen reduction than larger vessels (200–500 μm), while both small and large-diameter vessels exhibited similar degrees of remodeling in IPF-PH (76).

In our experience and in most pathologic studies of PH in lung disease including IPF-PH, COPD-PH, PH in other forms of nonspecific interstitial pneumonitis, or hypoxic PH in the setting of high altitude or sleep apnea, the presence of plexiform lesions has not been found (29, 59). Based on the cornerstone description of the pathology of PAH (72), we define plexiform lesions as an abnormal growth of ECs with EC markers (i.e., Factor 8 related antigen, VE-cadherin, or CD31) (199) and VEGF signaling molecules (196). However, we acknowledge that a standardized definition of plexiform lesion is lacking both in PAH and in descriptions of pulmonary vascular remodeling in the setting of Group 3 PH, with some calling abnormal luminal growth representative of plexiform lesions (49). It should also be noted that recent studies demonstrate that at least certain subtypes of plexiform lesions are the result of shunt-type lesions that connect pulmonary arteries to the bronchial circulation (207, 221) which may be late manifestation of end-stage PAH rather than an inciting factor in the pathogenesis of PAH. In addition, in Group 3 PH, we have not observed concentric lesions, which would typically be composed of ECs (203). As illustrated in Figure 4, the adventitia merges with fibrotic and inflammatory processes involving alveolar septa and bridging with airways. This “fibrotic reticulum” may eventually connect with the subpleural fibrotic areas (33).

The paradigm of vascular rarefaction that has been raised to explain the increased PVR in Group 1 PH has also been presented in IPF-PH and COPD-PH (197). Studies of COPD-PH have shown pruning of the small pulmonary vessels, causing decreased capillary density (23, 218) and correlating with severity of PH (23). Evidence for vascular rarefaction in fibrotic lung diseases has also been supported by gene expression data (37) and as well as by experimental animal studies (48). These studies, however, fall short because they did not rely on lung stereology, the gold standard for the quantification of histopathological features in the lung. It is not appropriate to compare fibrotic to nonfibrotic areas in terms of the vascular density, as these are divergent tissue types, with the single commonality that they occur in the same lung. Importantly, the studies did not consider the volume of reference tissue (i.e., the volume of fibrosis and alveolar airspace and septal tissues), which further undermines the validity of the findings.

We postulate that in IPF and perhaps in other forms of lung disease-associated PH, the process of remodeling and pathobiology begins on the “outside” (adventitial) but progresses to “inside” (luminal) injury and remodeling processes, highlighting the potential

contribution of fibrotic processes and inflammation in its pathobiology, as we will highlight below. This contrasts with the marked involvement of the intima in PAH, which is suggestive of an “inside to outside” order of pathobiological events, where the adventitia then becomes a later site of remodeling in PAH and conduit of inflammation that contributes to PAH (178). In conclusion, while there are likely differences in the pathology of Group 1 and Group 3 PH, more rigorous studies are needed to confirm and establish the significance of these findings.

Hypoxic vasoconstriction and pulmonary vascular remodeling

Hypoxia is sensed via carotid body chemoreceptors (triggering the hypoxic ventilatory response), oxygen sensors in the pulmonary vasculature (triggering hypoxic vasoconstriction), and oxygen sensors in various other tissues, resulting in activation of vascular endothelial growth factor in the heart and brain and erythropoietin in the kidney and liver (74, 77). In the pulmonary vasculature, hypoxia inhibits oxygen-sensitive potassium channels. This causes depolarization of pulmonary artery SMCs and activation of voltage-gated calcium channels, which results in calcium influx and Rho kinase-mediated pulmonary artery SMC vasoconstriction. Consequently, PVR and pulmonary arterial pressure increase (67, 121). The degree of pulmonary arterial pressure elevation increases with magnitude of altitude or hypoxia and exercise (134). Sustained and exaggerated pulmonary vasoconstriction over time is thought to cause pulmonary vascular remodeling which in turn contributes to ongoing elevation in PVR (104). Remodeling may occur in part as ECs sense humoral and hemodynamic changes, which trigger vasoactive and mitogenic factor production that alter pulmonary artery SMC function, proliferation, and apoptosis (150). Additionally, there is a shift in ECs to production of endothelin, thromboxane A2, and other vasoconstrictive substances (15, 21, 53, 186).

Animal models suggest that pulmonary vascular remodeling due to pure hypoxia is rapid in onset and only partially reversed by removal from hypoxia (177). Rabinovitch and colleagues demonstrated the presence of smooth muscle-like cells in normally nonmuscular pulmonary arterioles after exposing rats to hypoxia (143, 144). Many mechanisms including recruitment of progenitor cells, distal migration of resident smooth muscle stem cells, and pericyte recruitment have been implicated in this process (40, 45, 55, 164, 177, 217, 233). The onset of neomuscularization is rapid, with changes observed within hours of hypoxic exposure and peaking at 1 week (171). The proportion of muscularized pulmonary arterioles corresponds with increasing pulmonary arterial pressure (142). With the return to normoxia, pulmonary arterial pressure decreases but does not normalize, and some structural changes persist (143, 171, 177).

As with Group 1 PH, the initiating events in the development of PH in lung disease are unknown. Although SMCs comprise most of the remodeled parts of COPD and COPD-PH pulmonary arteries, ECs are implicated in the early stages of pulmonary vascular remodeling and PH development. Cigarette smoke can directly injure ECs, and EC dysfunction contributes to emphysema development and also causes vascular stiffness due to impaired release of the endothelium-derived vasodilating agents nitric oxide (NO) and prostacyclin (189, 201). Smokers without obstructive lung disease have reduced expression of endothelial

nitric oxide synthase (eNOS) (10). Hypoxia-induced EC damage can lead to over-production of the potent vasoconstrictors thromboxane A2 and endothelin-1 (ET-1) (135), which cause the neighboring SMCs to proliferate and vasospasm (187). ECs have been shown to influence pulmonary artery SMC inflammation in an autocrine manner and proliferation, hypertrophy, and collagen synthesis in a paracrine manner (110).

Gaps in understanding the pathobiology of Group 3 PH

The hypothesis that fibrosis-mediated vascular destruction and hypoxic vasoconstriction are the sole contributors to PH in IPF or severe emphysema is being increasingly challenged based on two important observations. Studies in animal models of COPD have shown that pulmonary vascular remodeling and PH precede the development of emphysema (162). Moreover, even patients with mild to moderate COPD without PH develop intimal thickening (113), as do smokers without COPD (158), caused by SMCs and deposition of elastic and collagen fibers (158). This may explain why pulmonary arterial wall thickness has not consistently been correlated with mean pulmonary arterial pressure (99, 226). Similarly, clinical data suggests that in IPF patients with similar levels of pulmonary functional impairment, some individuals develop PH while others do not (60, 122, 156). While the degree of vascular remodeling correlates with severity of regional fibrosis, studies of IPF pathology have also observed vascular remodeling in nonfibrotic regions of IPF lungs (132). These structural alterations ranged from thickening of the vascular smooth muscle layer to proliferative intimal lesions, to complete occlusion of the vessels in an appearance similar to the plexiform lesions in PAH (132), although not meeting our definition as described above. Cumulatively, this suggests that mechanisms other than fibrosis-mediated vascular destruction and hypoxic vasoconstriction occur in IPF-PH and are significant contributors to IPF-PH pathogenesis. This justifies a focus on mechanisms of pulmonary vascular remodeling in IPF-PH as well as Group 3 PH in general.

Many of the hypothesized mechanisms of pulmonary vascular remodeling in IPF or IPF-PH have been inferred from PAH pathophysiology, although as we have noted, these exact mechanisms of pulmonary vascular remodeling are unknown. Given the distinct differences in potential etiologic mechanisms that separate COPD, IPF, and PAH, it does not seem far-fetched to hypothesize that there are likely divergent mechanisms in the pathogenesis of PH in these different forms that could be detected using currently available techniques, such as with molecular profiling, but these studies have produced other surprising findings. In a study in which whole genome microarray analysis was performed on laser capture microdissected pulmonary artery profiles from COPD-PH, IPF-PH, and control donor lung explants, there was only a small overlap of differentially regulated genes between COPD-PH and IPF-PH pulmonary artery profiles compared to control lungs, while the majority of differentially expressed genes varied, with the largest observed differences seen between COPD-PH and IPF-PH in genes belonging to the retinol metabolism pathway and the ECM-receptor interaction pathway (76). The top up-regulated genes in the COPD-PH pulmonary artery profiles were then analyzed compared to donors with COPD without PH, and interestingly, no significant differences were found between these two groups. Furthermore, the severity of PH defined as increasing mPAP only correlated with minor changes in gene regulation across all samples including both COPD-PH and IPF-PH. A parallel comparison

of IPF-PH with IPF lungs without PH could not be performed in this study due to the lack of access to non-PH IPF lungs; however, in an earlier study by a different group using similar laser capture microdissection techniques, the gene expression of pulmonary arteries in IPF patients with and without co-existing PH was compared to that of controls (133). The study demonstrated that factors associated with SMC and EC proliferation, Wnt signaling, complement system activation, and apoptosis were differentially expressed in the small pulmonary arteries of IPF patients compared to controls, but interestingly, unsupervised and supervised clustering analyses revealed similar gene expression in IPF-PH and IPF non-PH arterioles. Thus, pathways involved in aberrant apoptosis and vascular proliferation are already activated in IPF patients without PH. Combined, these studies highlight the likelihood that patients with COPD and IPF may develop PH through divergent mechanisms, and that much like the histopathologic changes that are seen even before the clinical development of PH, reprogramming of the transcriptome could also occur before the appearance of PH in the setting of ILD or COPD.

Inflammation in Group 3 PH

Inflammatory pathways play a central role in the development of hypoxia PH (177). Hypobaric hypoxia increases transforming growth factor β (TGF-β) secretion by primary pulmonary adventitial fibroblasts and macrophages (100). TGF-β appears to promote the Nox4 isoform of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, leading to the production of endogenous reactive oxygen species and pulmonary artery SMC proliferation (8, 183). Additionally, hypobaric hypoxia increases levels of pulmonary artery SMC IL-6, activating inflammatory responses and fibrosis. IL-6 deficient mice exposed to hypoxia demonstrate less macrophage recruitment, pulmonary vascular remodeling, and PH (115, 161). Other inflammatory pathways triggered by endoplasmic reticulum stress and mediated by GRP78, PERK, IRE1, ATF4, ATF6, CHOP, and caspase-12 may also contribute to hypoxic pulmonary vascular remodeling in subacute to chronic hypoxia (139). Finally, macrophages appear to play a role in pulmonary artery SMC proliferation (232). Hypoxia induces the production of macrophage migration inhibitor factor (MIF) leading to release of inflammatory mediators and stimulation of pulmonary artery SMC proliferation contributing to PH (168, 238).

Thus, despite a focus on ECs and pulmonary vascular smooth muscle cells (PVSMCs) in PH/PAH, many recent studies support a causative role for immune cells in PH/PAH (3, 54, 160, 199, 231). Pulmonary macrophages accumulate in PH lung tissues, and their presence is associated with worsened PH pathology (54, 100, 231). To define mechanisms underlying this observation, recent studies using PH hypoxia-induced models demonstrated that infiltrating pulmonary macrophages promote pulmonary vascular remodeling and PH (231). This data suggests that interactions between macrophages and vascular structural cells (ECs, PVSMCs, and fibroblasts) drive vascular remodeling and PH pathogenesis. Extending this observation to human IPF-PH, recent data demonstrate the presence of pulmonary macrophages around remodeled vessels. This finding suggests a causal role for pulmonary macrophages in IPF-PH development and progression, though the precise mechanisms are presently unknown.

Inflammation, which plays an important role in the pathophysiology of COPD, is also implicated in the development of pulmonary vascular remodeling and PH in COPD. Overexpression of protease-activated receptor 2 (PAR-2), whose gene expression is upregulated in ECs by cytokines, in cigarette smoke-exposed mice not only developed emphysema, but also developed muscularization of small intrapulmonary vessels; these mice also had an imbalance between vasoconstrictors (ET-1) and vasodilators (VEGF, NO synthase, and inducible iNOS) as well as increased production of growth factors involved in vascular cell proliferation (41). Myeloid-cell-specific deletion of iNOS in mice was found to ameliorate the increase in expression of CD206 on interstitial macrophages and protect against the development of PH in smoke-exposed mice (65). Furthermore, CD206-positive and iNOS-positive macrophages were seen to accumulate in the proximity of remodeled vessels in the lungs of COPD patients (65). More investigation into the crosstalk between immune cells and the other cell types in the pulmonary vasculature is required to determine whether there are targetable inflammatory pathways in the pathogenesis of Group 3 PH.

New Strategies for Defining the Molecular Pathobiology of Pulmonary Hypertension Across All Groups

While the classification of PH according to clinical characteristics has been useful in advancing the care of PH patients, it has been a precarious platform from which to discover new treatments and to build a framework to understand the complex molecular pathophysiology of numerous PH phenotypes. The vast majority of patients with PH, predominantly those with Group 2 PH due to left heart disease and Group 3 PH due to chronic lung disease, have been excluded from being able to benefit from most currently available treatments, while the search for new therapies for these groups has been largely unfruitful. Other patients currently grouped under Group 1 PH may be inappropriately included and could benefit less or even be harmed by the initiation of pulmonary vasodilator therapy, particularly in the case of patients with pulmonary veno-occlusive disease (PVOD). Big data sets generated by high-throughput molecular techniques and machine learning algorithms can be used to address the limitations of these reductionist approaches and to disentangle the complex relationships between genotype, endophenotype, and clinical phenotype of PH.

In a study of human explanted lungs from patients with PAH, PVOD, COPD, and IPF, RNA analysis of targeted sampled areas revealed that PAH and PVOD samples exhibited the greatest intergroup differences in gene expression, while pathway analysis revealed that PVOD patients shared 11-fold more similarities with patients with IPF compared to with PAH (128). Another study using laser capture microdissection of pulmonary artery profiles combined with unbiased whole genome microarrays revealed distinctly different gene expression patterns and disturbed regulatory pathways between IPF-PH and COPD-PH, with retinol metabolism and ECM receptor interaction pathways being the most affected processes (76).

A deeper understanding of disease pathogenesis using genomics, proteomics, metabolomics, and epigenetics is needed to identify novel therapeutic targets and disease-specific

biomarkers for all forms of PH. Unbiased algorithms and clustering techniques can allow for the discovery of mechanistic pathways in an agnostic fashion. The 2010 NHLBI Pulmonary Vascular Strategic Plan identified using “omics” technologies and a systems approach, combining institutional cohorts and integrating multidimensional omics, clinical, and outcome data, as top priorities in pulmonary vascular research (73). These approaches have the potential to (i) reveal molecular signatures that could be targeted with new precision methods, (ii) deeply characterize endotype-genotype-phenotype profiles to predict which patients are at greatest risk of progression and to discover signatures of treatment response, and (iii) identify using high-throughput screening molecules and repurposed drugs that could target regulatory pathways in PH. Thus far, these approaches have largely been used to understand more deeply the pathogenesis of PAH. While they have furthered the understanding of lung diseases such as COPD and IPF, PH in association with these diseases has not been studied with such precision.

Genomics

Using new sequencing techniques, several PAH-related genes have been identified in recent years with significant implications for new treatment strategies. Mutations of bone morphogenic protein receptor-2 (*BMPR2*), a member of the TGF-β superfamily were identified as the underlying cause of 80% of familial PAH and are involved in 25% of sporadic PAH cases (112). This was followed by discoveries of mutations in *ACVRL1* (encoding activin A receptor type II-like 1) and *ENG* (encoding endoglin) in hereditary hemorrhagic telangiectasia, a syndrome complicated by the development of PAH, two proteins that also participate in BMPR2 signaling through dimerization (69, 193). Other mutations have been identified in transcription factors SMAD8, SMAD4, TBX4, and SOX17 which are downstream of BMPR2 signaling (124), and BMP9 (also known as growth differentiation factor 2 *GDF2*), the preferred ligand for the BMPR2 complex (63). The loss of BMPR2 functional signaling results in the loss of antiproliferative signaling, leading to EC dysfunction and the characteristic pulmonary vascular remodeling in PAH. The discovery of mutations in BMPR2 and genes related to BMPR2 signaling has led to the first phase 2 trials of drugs targeting this novel pathway. Sotatercept, a novel fusion protein which acts by sequestering excess ActRIIA ligands, reducing and thereby rebalancing ActRIIA-Smad2/3 pro-proliferative with BMPR2 antiproliferative signaling, was shown to reduce PVR in patients already receiving background PAH therapy after 24 weeks of treatment, and is now being tested in phase 3 clinical trials (79). A trial of the calcineurin inhibitor FK506 (tacrolimus), which upregulates BMPR2 expression, was shown to be safe and well-tolerated in PAH patients (174, 175). Preclinical trials have shown that administration of BMP9 has the potential to reverse PAH in a mouse model of PAH bearing a heterozygous knock-in of a human BMPR2 mutation, *R899X*, and in two severe rat models of PAH (111). Interestingly, one study has shown that BMPR2 was also significantly decreased in the lung tissue and macrophages of IPF patients with a greater decrease in IPF-PH group (30), raising the question of whether these therapies targeting BMPR2 expression and signaling could be applied in IPF-PH.

Epigenomics

The importance of epigenetic mechanisms in the development and progression of PAH has become increasingly clear and present an opportunity for new therapeutic targets, including dysregulation of noncoding RNA networks, histone acetylation and methylation, and bromodomain proteins, all of which act as epigenetic regulators of pulmonary vascular wall structure and function. Many publications have demonstrated the importance of microRNAs in PAH, with notable examples being miR-140-5p, which can upregulate the BMPR2 signaling pathway and prevent development of PAH *in vivo* (154), miR-126 and miR-223 of which downregulation can contribute to development of RV failure in PAH (137, 165), and the miR-130/301 family, which was shown to be a master regulator of cellular proliferation in PAH (16). Additional studies have implicated miR-124 as playing a crucial role in controlling the metabolic and proliferate state of ECs, SMCs, and adventitial fibroblasts (236, 237). DNA methylation patterns in the mitochondrial superoxide dismutase-2 (SOD2) gene, the major generator of H_2O_2, were found to create proliferative, apoptosis-resistant pulmonary artery smooth muscle cells (PASMCs) that led to the initiation and progression of heritable PAH in the Fawn hooded rat model of PAH, and reversal by DNA methyltransferase inhibitor 5-*aza*-2′-deoxycytidine restored both SOD2 expression and the ratio of proliferation to apoptosis (6). Histone deacetylases (HDACs) and their inhibitors have been studied with great interest for their therapeutic potential, as the use of small inhibitory molecules such as valproic acid and suberoylanilide hydroxamic has been shown to successfully prevent and partially reverse PAH in rats and inhibited platelet-derived growth factor-stimulated proliferation of fibroblasts and human vascular SMCs *in vitro* (101, 239). Significant effects of HDAC inhibition on ventricular remodeling in PH have also been reported (25, 224). The bromodomain and extraterminal-containing (BET) protein family, which includes BRD2, BRD3, and bromodomain-containing protein 4 (BRD4), are epigenetic reader proteins that bind to specific acetylated protein residues on histone tails where they facilitate the assembly of transcription complexes and are receiving much attention in cardiovascular disease (19, 109). Upregulation of BRD4 has been found in distal pulmonary arteries and PASMC of PAH patients, and pharmacologic BRD4 inhibition has been shown to reverse experimental PAH (116). A phase 2 clinical trial using the BRD4 inhibitor apabetalone is currently in progress. Lastly, germline mutations of TET2, encoding ten-eleven translocation (*TET*) methylcytosine dioxygenase 2, a key enzyme in DNA methylation, were recently reported in a large cohort of patients with PAH (136).

Proteomics

Targeted, liquid chromatography and mass spectrometry-based approaches, or targeted antibody/aptamer-based approaches, can be used to generate large data sets with expression patterns of >1000 targets from a single sample. In-tandem liquid chromatography-mass spectrometry performed on whole lobe homogenates from healthy and PAH explanted lungs found differences in 25 proteins between the groups, with increased expression of chloride intracellular channel 4, a multifunctional protein involved in numerous processes implicated in PAH pathogenesis including angiogenesis, and TGF-β, endothelial growth factor, and bone morphogenetic protein signaling (1). A plasma proteomics approach using an aptamer-based assay of 1129 plasma found 20 proteins that differentiated survivors and nonsurvivors in an observational cohort of idiopathic and heritable PAH patients,

with a panel of 9 proteins that provided prognostic information independent of plasma NT-proBNP concentrations and independent of the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL) risk score (Figure 5) (14, 153). This protein panel score was then validated in another independent PAH cohort, with an increasing score distinguishing low-, medium-, and high-risk groups (153). More recently, complete proteomic analysis of the plasma of patients with PAH and healthy individuals found higher levels of netrin-4, while higher thrombospondin-2 levels had a protective effect, thus identifying pathways that if inhibited may prevent development of PAH and or if potentiated could decrease the severity of established PAH (68).

Metabolomics

One of the early insights that supported the cancer hypothesis of PAH was the observation of a switch in IPAH cells from aerobic metabolism to glycolysis (e.g., the Warburg effect), as had previously been observed in solid tumor malignancies in low oxygen environments (198). A complex picture of the interplay between cells of the pulmonary vasculature and the cells in the immediate microenvironment has since emerged, with a role for metabolic reprogramming of resident and recruited cells influencing pulmonary vascular remodeling and other hallmarks of PH including proliferation, resistance to apoptosis, inflammation, and fibrosis (38). Continued interest in energy metabolism and new metabolomics approaches including nuclear magnetic resonance (MR) and liquid chromatography-mass spectrometry have further advanced the understanding of the changes in metabolism in PH. Layered transcriptomic and metabolomic analyses of human pulmonary microvascular ECs expressing two different disease-causing mutations in BMPR2 confirmed the previously seen increase in aerobic glycolysis but also uncovered widespread metabolic changes, with significant upregulation of the pentose phosphate pathway, increases in nucleotide salvage and polyamine biosynthesis pathways, decreases in carnitine and fatty acid oxidation pathways, and major impairments of the tricarboxylic acid (TCA) cycle and failure of anaplerosis, some of which were confirmed to be present in the serum of PAH patients (52). A study using targeted mass spectrometry to perform metabolic profiling of 71 patients who also underwent right-heart catheterization and radionuclide ventriculography found an association of hemodynamic indicators of RV-pulmonary vascular dysfunction with 21 metabolites including circulating indoleamine 2,3-dioxygenase (IDO)-dependent tryptophan metabolites (TMs), TCA intermediates, purine metabolites, and arginine-nitric oxide metabolic pathway constituents (107). A comprehensive study of plasma metabolites in a large cohort of IPAH or heritable PAH patients (n = 365) found 51 metabolites that were able to distinguish PAH from healthy control subjects, with a subset of these metabolites also able to distinguish PAH from control subjects with other diseases (151). These included tRNA-specific modified nucleosides (N_2,N_2-dimethylguanosine, N_1-methylinosine), TCA cycle intermediates (malate, fumarate), glutamate, fatty acid acylcarnitines, tryptophan, and polyamine metabolites and decreased levels of steroids, sphingomyelins, and phosphatidylcholine. Moreover, 62 metabolites were prognostic of death in PAH, with half of these independent of established prognostic markers. Interestingly, patients who responded to calcium channel blocker therapy had metabolic profiles similar to those of healthy control subjects, suggesting possible applications of these methods to improve risk stratification and identify responders to treatment. An extension of

this study to patients with Group 4 PH (or CTEPH) found that untargeted analysis of plasma metabolites was able to distinguish CTEPH from control patients and that many metabolic changes were similar between CTEPH and IPAH patients, with only five metabolites able to distinguish CTEPH from IPAH (185). Another investigation into the intracellular metabolism of ECs in PH patients (which included IPAH, connective disease-associated PH, as well as CTEPH) demonstrated again a clear separation between plasma metabolites of PH patients and controls, and furthermore revealed four distinct intracellular EC metabolic clusters in patients with PH, with notably different disease severity between the clusters (24). Metabolic profiling studies thus have the potential to discover new biomarkers, pathways for further characterization and intervention, and commonalities between types of PH that could be targeted with therapeutic interventions (Figure 6).

Machine learning

ML algorithms offer a computational platform to identify patterns in big data sets generated by high-throughput technologies. Although a proper understanding of their limitations and proper application of these algorithms is needed to avoid common pitfalls, recently summarized in a review by Rhodes et al. (Table 3) (152), they offer a unique opportunity to identify patterns in large omics data that can elucidate pathobiological pathways in PH (47), identify new etiological sub-populations within PH patients (129, 184, 222), reclassify types of PH using newly revealed commonalities, develop risk stratification calculators to guide clinical decision making (47, 90, 91, 105, 152), and eventually suggest novel therapeutic compounds (96).

There are two types of ML algorithms:

1. Supervised algorithms (e.g., decision trees and random forests of decision trees, artificial neural networks, multivariate logistic regression) are applied when training a model to recognize a known event or estimate a specific measurement. For example, if 1000 proteins are measured in 500 patients with PAH and 500 patients without PAH, a supervised ML model can be “trained” to identify a pattern in those 1000 proteins that detects whether a patient has PAH. This is referred to as a “classification” model. If the objective was to use these 1000 proteins to estimate a continuous variable (e.g., mPAP, PVR), supervised ML models can also be trained for that purpose, which is known as a “regression” model. These kinds of models can be very useful for creating a diagnostic algorithm, estimating invasive parameters through imaging or blood markers (89, 90), or estimating prognostic risk stratification scores (12, 47, 85, 91). Supervised ML was used to train an algorithm using electronic health record healthcare utilization data from PAH patients in England to preemptively predict the development of idiopathic PAH, which was cross-validated in a subgroup of the cohort not used in the training, and was found to have excellent specificity and negative predictive value (92). Another outcome risk assessment model based on Bayesian analysis outperformed both the REVEAL (13) and REVEAL 2.0 calculators in the prediction of 1-year survival on the same cohort and external validation cohorts (85). However, assumptions about similarities and differences based on traditional PH categories and misclassification can result

in bias and limit the detection of important similarities and differences in supervised ML.

2. Unsupervised algorithms (e.g., principal component analysis, *k*-means clustering) are applied to data to detect patterns when not looking for a specific event. For example, if 1000 proteins were measured in proteins in 1000 PAH patients along with their phenotypic attributes, unsupervised ML algorithms could tell if these patients cluster into sub-groups. These algorithms have been used in other diseases to reclassify and uncover subgroups, such as in a study of patients with heart failure with preserved ejection fraction (HFpEF), in which 67 phenotypic variables were analyzed and classified patients into three groups that had unique clinical characteristics, cardiac structure and function, invasive hemodynamics, and outcomes (163). Another study utilized exercise profiles using data from pulmonary function testing, cardiopulmonary exercise testing, and metabolic data to define four exercise groups with different variables that predicted hospitalization in patients with exercise intolerance (130). In studies using unsupervised ML in PAH, Sweatt et al. (184) utilized an unsupervised ML algorithm to explore if circulating markers of inflammation could identify distinct sub-populations of PAH patients and discovered four distinct immune phenotypes distinct from clinical subtypes which had differing clinical risk metrics and long-term survival. Other researchers utilizing similar techniques on whole blood transcriptomics identified that PAH patients fell into three subgroups based on up- and down-regulation of certain genes that were associated with poor, moderate, and good prognosis (86). In summary, unsupervised ML has great potential to reclassify diseases, allowing patients with shared perturbations in genomic, proteomic, or metabolic profiles to be partitioned into subgroups in an agnostic data-driven manner. However, clustering by unsupervised ML is highly sensitive to confounding sources of heterogeneity, such as comorbidities, medications, and batch effects, thus it is critical that any investigators using these techniques carefully consider their cohort selection and ensure data quality control before data processing.

ML algorithms can identify patients at greatest risk that would potentially benefit from more aggressive up-front therapy, and to identify those most likely to respond to therapy. Currently, patients with a new diagnosis of PAH undergo right heart catheterization to obtain hemodynamic measurements and to determine vasoresponsiveness to inhaled nitric oxide, which predicts a good long-term response to therapy with calcium channel blockers (169). ML offers more opportunities to identify other biomarkers indicative of potential treatment response and to measure early treatment response or failure. A recently concluded phase 2 trial evaluating the effect of rituximab B-cell depletion therapy in systemic sclerosis (SSc)-PAH utilized supervised ML models to identify baseline biomarkers that predicted a clinical response at 4 weeks (235). Training of each model began with 168 input variables including cytokines, chemokines, factors, immunoglobulin subclasses, autoantibodies, B-cell subsets, and clinical features, involved iterative algorithm resampling runs and recursive feature elimination to select variables subsets that best classified clinical responders, and identified that low concentrations of rheumatoid factor, IL-12, and IL-17 were consistently

found to be favorable predictors of rituximab response (receiver operating characteristic area under the curve 0.88–0.95) (235).

While grouping and risk stratification using machine learning models requires validation in independent cohorts, future trial designs should increasingly incorporate these methods to optimize patient selection, and once validated, treatment response signatures can be used to guide therapeutic decisions.

Lessons learned from the implementation of these big data approaches combined with machine learning could allow the field of pulmonary vascular disease (PVD) research to benefit from emerging techniques which are already being successfully employed across a spectrum of disciplines. However, due to the rare nature of PAH and the relatively small size of clinical registries compared to more common diseases, laboratories studying pulmonary vascular disease have applied unsupervised (86, 184) and supervised (128) machine learning techniques to datasets with only a few hundred observations, compared to much larger data sets that have been used in other fields. As a result, while these innovative studies are exposing the PVD community to exciting new ways of analyzing high-throughput data, their findings will need to be validated across different datasets and with different techniques before seriously challenging the central dogma of PVD.

We propose that adoption of machine learning technology and advances could be made by offering the PVD research community access to data from across multiple centers added to a publicly available growing body of records, which could become the gold standard for validation. This would ensure that any laboratory generating data for an ongoing study would: (i) Be more likely to use established data acquisition methodology that could be validated with overlapping markers before publication; (ii) Offer researchers a quantitative comparison of their results with established datasets, which would serve as a simultaneous validation of the acquisition and analysis pipeline; and (iii) Prevent saturating the literature with underpowered and unvalidated results.

Discovering novel drug therapies

High-throughput screening (HTS) is a drug discovery process that allows automated testing of large numbers of chemical and/or biological compounds for a specific biological target. This process has been used successfully in discovering new treatments for rare diseases. Treatment of cystic fibrosis (CF), a disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which previously shortened patient life expectancy by decades, has been revolutionized by the use of HTS to identify small-molecule potentiators (208, 228) resulting in the development of drugs that are now available to treat 90% of known genetic mutations in CF (11). Artificial intelligence (AI) technologies provide further promise of rapidly speeding up the process of identifying and testing candidate drugs. Traditional HTS methods begin with a screening library of up to 1 million compounds, with each costing US$50 to $100 for a total cost of several million U.S. dollars, followed by years of compound optimization and identifying preclinical drug candidates, while AI can assist in all steps of the pipeline for new drug development (26). A virtual compound library of several billion molecules can be screened within a few days, followed by a few months to a year to identify preclinical drug candidates using AI to

predict the physical properties of drugs in terms of bioavailability, bioactivity, and toxicity, 3D structures of target proteins, drug-protein interactions, and to perform retrosynthesis pathway prediction as a method for designing the organic synthesis of a drug candidate (7, 214). A deep learning model was trained to predict molecules with antibacterial activity and discovered a novel antibiotic, halicin, that demonstrated bactericidal activity against a diverse spectrum of pathogens including *Mycobacterium tuberculosis* and multi-drug resistant Enterobacteriaceae and Acinetobacter, as well as eight other antibacterial compounds that are structurally unique compared to all known antibiotics (181). Combining insights into the molecular mechanisms underlying the pathogenesis of PH with high-throughput drug screening techniques with assistance from AI has the potential to discover new categories of treatments for this disease at a much more rapid pace than has been seen to date in the field of pulmonary vascular disease.

Conclusions

Despite significant advances in the understanding of pathogenesis of PH and its treatments, significant gaps remain in understanding the initiating factors and the role of pathologic lesions in PAH, and strides must be made to standardize the techniques and definitions applied to lung pathology in both Group 1 and Group 3 PH. Advances in scientific technologies have led to deeper, richer understandings of the genetic and pathobiologic basis of pulmonary vascular disease and have revealed similarities between as well as heterogeneity within the currently defined WHO groupings of Group 1 and Group 3 PH. Greater emphasis remains on Group 1 PH or PAH, while the use of these technologic advances to understand the pathobiology of PH in lung disease as well as other types of PH currently excluded from the Group 1 PH classification remains limited. Leveraging the substantial gains made in understanding the genomics, epigenomics, metabolomics, and proteomics of PAH to understand the full spectrum of the complex, heterogeneous disease of all forms of PH is needed. Multimodal omics data as well as supervised and unbiased machine learning approaches after careful study design and selection of patients could lead to earlier diagnosis, precise risk stratification, predictions of disease response, new sub-phenotype groupings within types of PH, and identification of similar subgroups and pathways between PAH and other types of PH that could lead to new treatment targets. These insights combined with high-throughput drug screening technologies with the assistance of artificial intelligence could offer a more rapid pathway to the development of new therapies.

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Didactic Synopsis

Major teaching points

- New methodologies have demonstrated significant heterogeneity in the types of pulmonary vascular lesions observed in pulmonary arterial hypertension (PAH). They have also revealed both pathobiologic similarities as well as differences between PAH and other diseases in which pulmonary hypertension (PH) is observed, such as idiopathic pulmonary fibrosis (IPF).
- Patients with pulmonary hypertension secondary to lung disease (Group 3 PH) have equally poor if not worse mortality compared to those with pulmonary arterial hypertension (Group 1 PH) despite having lower pulmonary arterial pressure and right ventricular dysfunction, highlighting the urgent need to understand its pathobiologic underpinnings, which could lead to identification of new therapeutic options.
- At present, it remains unclear whether treatment of other groups of PH with the pulmonary vasodilator therapies used in Group 1 PH is appropriate or potentially harmful.
- While there are likely pathologic differences between Group 1 and Group 3 PH, application of rigorous techniques including lung stereology (i.e. quantification of three-dimensional anatomic abnormalities) and precise localization of vascular lesions (for example in relationship to areas of fibrosis) will help define the significance of these differences.
- Multimodal omics data as well as supervised and unbiased machine learning could lead to earlier diagnosis, more precise risk stratification, better predictions of disease response, new sub-phenotype groupings within types of PH, and identification of shared pathways between PAH and other types of PH that could lead to new treatment targets.

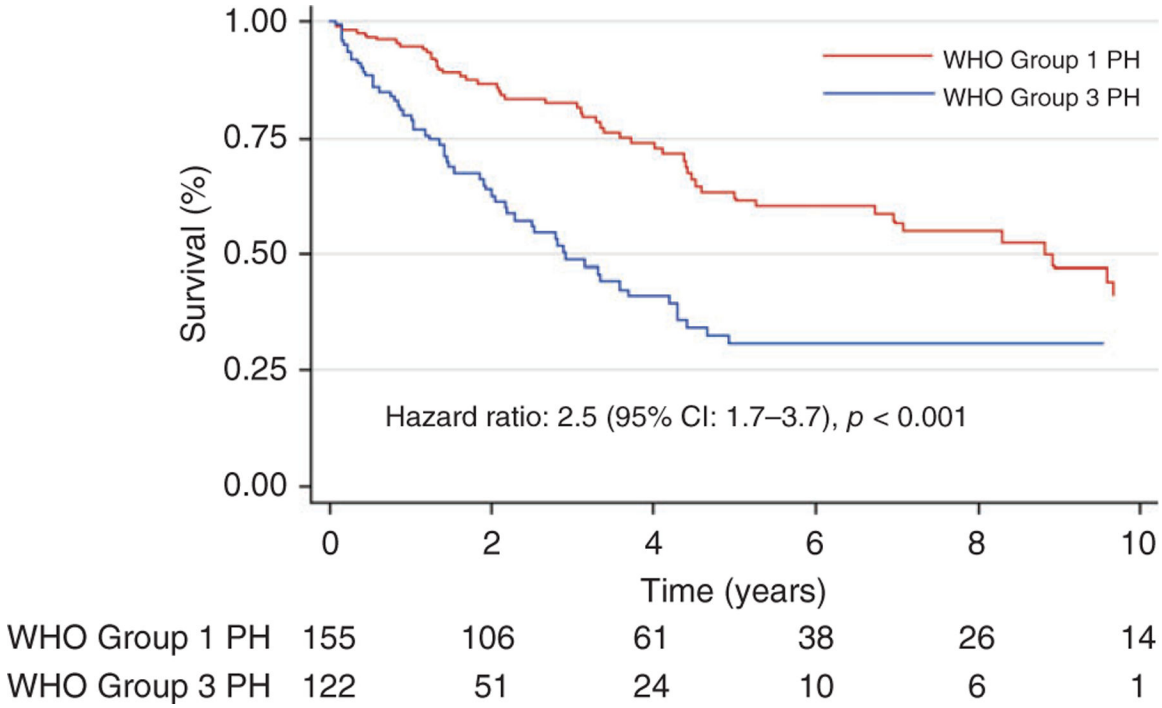

Figure 1.

Survival in Group 1 and Group 3 PH. Reused, with permission, from Prins KW, et al., 2018 (138), American Thoracic Society.

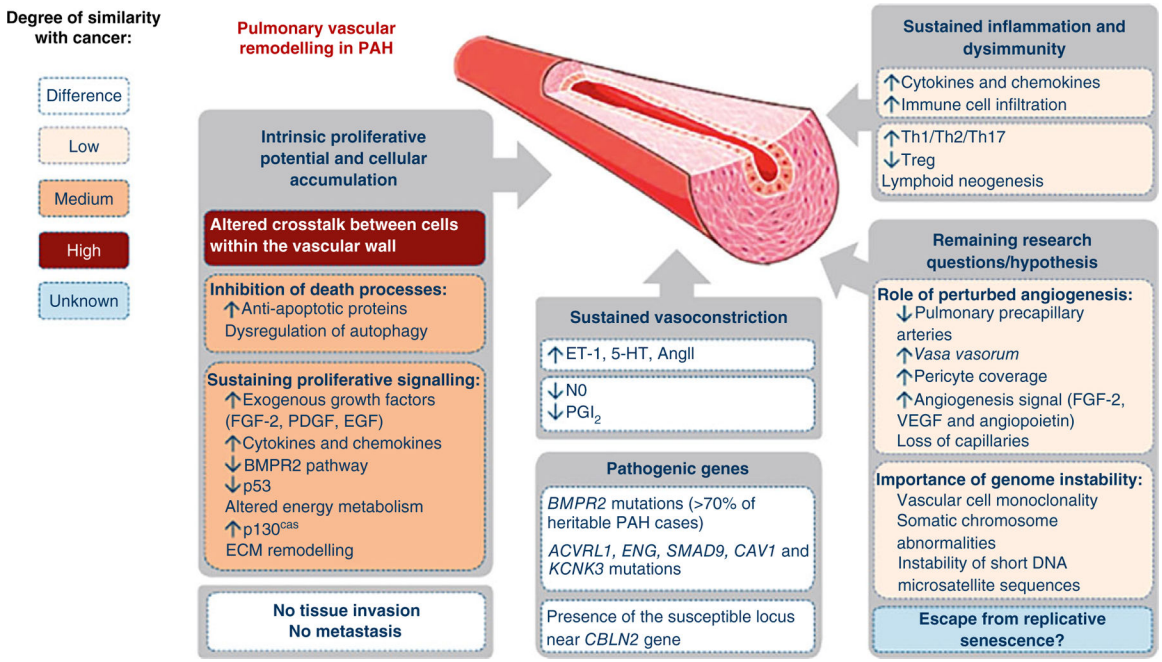

Figure 2.

Pathogenic concepts of pulmonary arterial hypertension: Differences and similarities with cancer. FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; BMPR2, bone morphogenetic protein receptor 2; ECM, extracellular matrix; ET-1, endothelin-1; 5-HT, serotonin; NO, nitric oxide; PGI2, prostacyclin; Th, T-helper cell; Treg, regulatory T-cell; VEGF, vascular endothelial growth factor. Reused, with permission, from Guignabert C, et al., 2013 (66), European Respiratory Society.

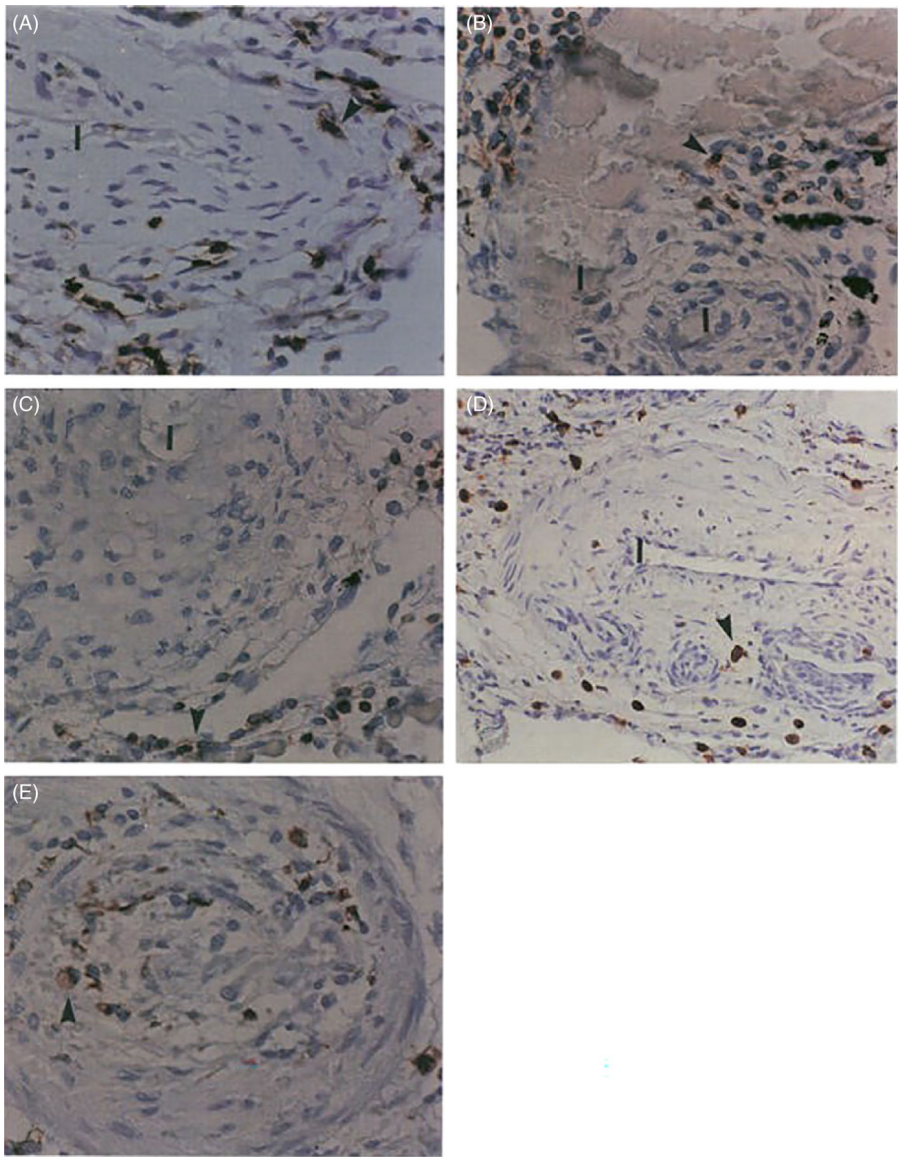

Figure 3.

Inflammatory components in plexiform type lesions of PAH. Inflammatory cells are highlighted by the anti-CD45 immunostaining (arrowheads) (A), B-cell marker (UL-26) (B), T-cell marker (UCHSC) (C), and macrophage marker (CD68) (D and E) (arrowheads). Note infiltration of the outer portion of the plexogenic vessel (D) and vessel obliterated by concentric proliferation (E) by macrophages (immunoperoxidase, A-C, E 500X; D: 200x). I-Vascular lumen. Reused, with permission, from Tuder RM, et al., 1994 (199), from Elsevier.

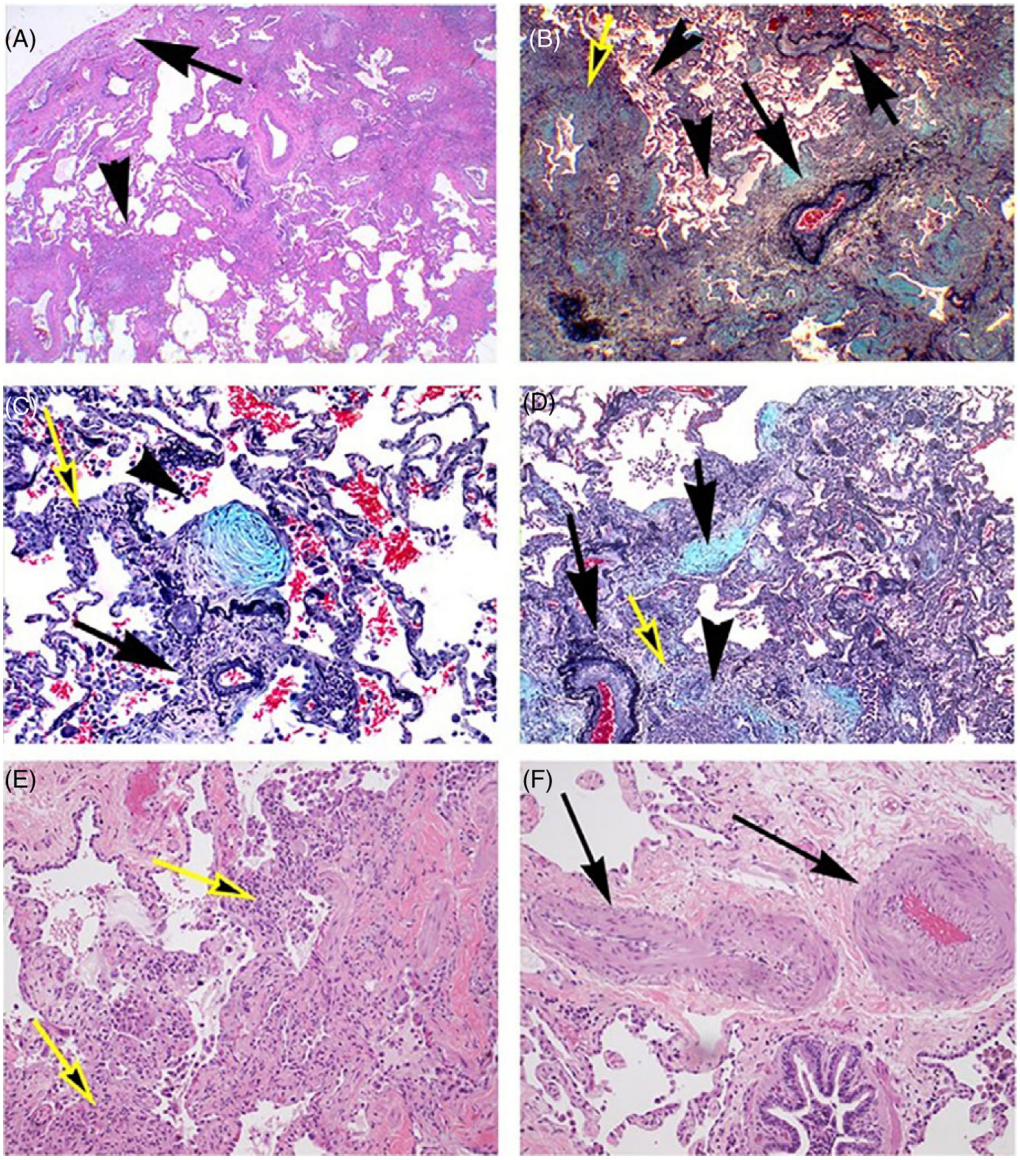

Figure 4.

Inflammatory and fibrotic processes in usual interstitial pneumonia with marked pulmonary vascular remodeling. (A) Low magnification of subpleural region showing marked subpleural fibrosis (arrow), extending irregularly toward central regions of the lung with conglomerates of lymphoid cells (arrowhead) (Hematoxilin and Eosin). (B) Marked perivascular (arrows) and airway (yellow arrow) fibrosis and inflammation, forming a continuous process along the bronchovascular and parencymal structures. The pulmonary arteries show intima thickening, with significant obliteration of the vascular lumina (Pentachrome). (C) Exudative fibroblastic focus (arrowhead) next to small pulmonary artery (arrowhead), accompanied by scattered inflammatory cells (Pentachrome). (D) Active bridging fibrosis along alveolar and bronchovascular (yellow arrow) associated with exudative process (arrow) and inflammation (arrowhead). Note the marked obliteration of an adjacent pulmonary artery (long arrow) (Pentachrome). (E) Active inflammation and

interstitial fibrosis in the advancing edge of usual interstitial pneumonia (yellow arrow) (Hematoxilin and eosin). (F) Marked media and intima thickening in muscular artery (arrows) in a region not immediately affected by UIP (arrows) (Hematoxilin and eosin).

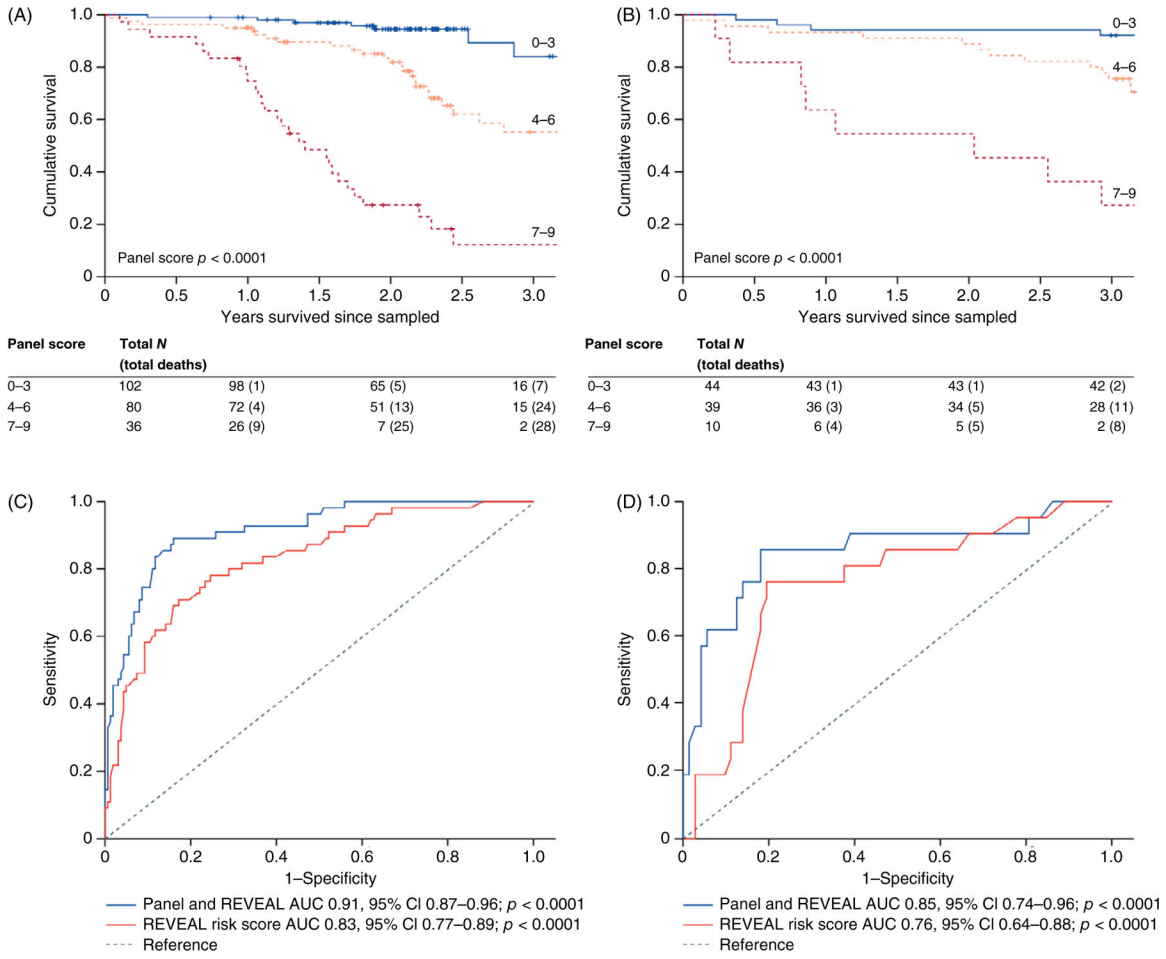

Figure 5.

Survival analysis of based on a panel of plasma proteins and REVEAL score in an observational cohort of idiopathic and heritable PAH patients. Kaplan-Meier survival estimates in patients with different panel scores, in all patients with idiopathic pulmonary arterial hypertension from (A) cohorts 1 and 2 and (B) cohort 4. ROC analysis of Cox models before and after addition of the prognostic protein panel to the established equation, in all patients with idiopathic pulmonary arterial hypertension from (C) cohorts 1 and 2 and (D) cohort 4. ROC, receiver operating characteristic; AUC, area under the curve. Reused, with permission, from Rhodes CJ, et al., 2017 (153)/Elsevier/CC BY 4.0.

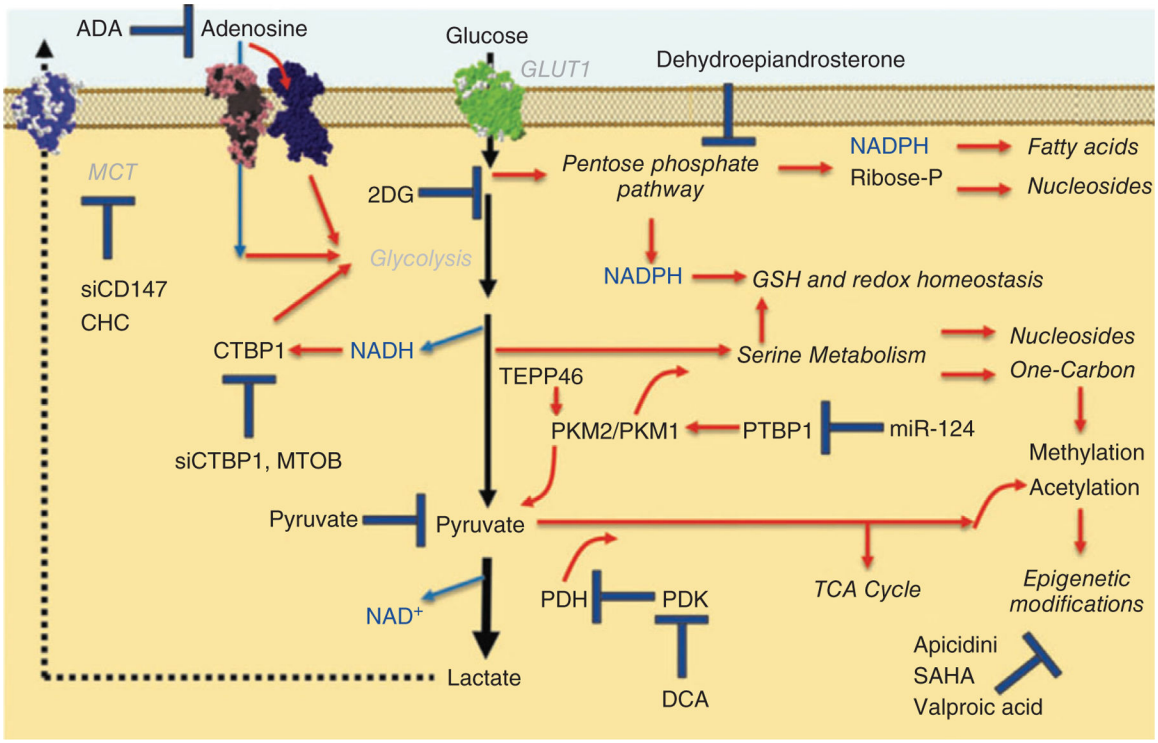

Figure 6.

Metabolic pathways and potential therapeutic interventions in PH. CHC, *a*-cyano-4-hydroxycinnamic acid; DCA, dichloroacetate; PDK, pyruvate dehydrogenase kinase. Reused, with permission, from D'Alessandro A, et al., (38)/with permission of Mary Ann Liebert.

Table 1

World Symposia on Pulmonary Hypertension—Classification of PH

Group 1 PH
Idiopathic PAH
Heritable PAH
Drug- and toxin-induced PAH
PAH associated with:
Connective tissue disease
HIV infection
Portal hypertension
Congenital heart disease
Schistosomiasis
PAH with long-term response to calcium channel blockers
PAH with overt features of venous/capillaries involvement (PVOD/PCH)
Persistent PH of the newborn syndrome
Group 2 PH due to left heart disease
PH due to heart failure with preserved LVEF
PH due to heart failure with reduced LVEF
Valvular heart disease
Congenital/acquired cardiovascular conditions leading to postcapillary PH
Group 3 PH due to lung disease and/or hypoxia
Obstructive lung disease
Restrictive lung disease
Other lung disease with mixed restrictive/obstructive pattern
Hypoxia without lung disease including high altitude
Developmental lung disorders
Group 4 PH due to pulmonary artery obstructions
Chronic thromboembolic PH (CTEPH)
Other pulmonary artery obstructions
Group 5 PH with unclear and/or multifactorial mechanisms
Hematologic disorders
Systemic and metabolic disorders

Others
Complex congenital heart disease

Definition of abbreviations: PAH, pulmonary arterial hypertension; PVOD, pulmonary vascular occlusive disease; PCH, pulmonary capillary hemangiomatosis; LVEF, left ventricular ejection fraction.

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Table 2

Targeting Signaling Pathways Involved in Cancer Biology for Controlling/Regressing Enhanced Proliferation, Survival, and Apoptosis Resistance of Lung Vascular Cells in Pulmonary Hypertension

Molecule/ Pathway	Cell Type	Status in PAH/Experimental PH	Function	Inhibitor(s)/ Activator(s) Tested	Effect of Inhibitor(s)/Activator(s)
RTKs					
PDGFR	PASMCs	PASMCs:	PASMCs:	Imatinib	Inhibits proliferation of human IPAH and rat MCT-PH PASMCs (219)
	PAECs	↑ Human IPAH	↑ Proliferation		Reverses MCT-PH in rats (61, 219)
		↑ Rat HPH	↓ Apoptosis		Showed efficacy in phase II (59) and III (196) trials
					Improves PVR and 6MWD in patients with severe PAH (196)
				Nilotinib	Reverses pulmonary vascular remodeling in rat MCT-PH and HPH (61)
EGFR	PASMCs	No significant alterations in experimental/clinical PH	PASMCs: ↑ Proliferation	PKI166	Mediates apoptosis in PA organ culture (98) Attenuates rat MCT-PH (98)
			↓ Apoptosis	Gefitinib	Attenuates rat MCT-PH (127) No significant benefits in mouse HPH (127)
				Erlotinib	Attenuates rat MCT-PH (127) No significant benefits in mouse HPH (127)
				Lapatinib	No therapeutic benefit in experimental PH (127)
FGFR	PAECs PASMCs	↑ Human IPAH (FGF-2) ↑ Rat MCT-PH (FGF-2, FGFR1)	PAECs: ↑ Proliferation PAECs: ↑ Proliferation	SU5402	Reverses rat MCT-PH (128)
Ras/Raf/ MAPK	PAECs	↑ Rat MCT-PH (p-Raf-1, p-ERK) ↑ Rat HPH ↑ Rat SuHx-PH (p-ERK, p-MEK1/2)	PAECs: ↑ Proliferation Cardiomyocytes: ↓ Vasopressin-induced hypertrophy	Sorafenib	Attenuates rat MCT-PH (81) Improves RV function in PAB rats (95) Attenuates rat HPH and SuHx-PH (56)
PI3K	PASMCs PAECs	Unknown	PASMCs: ↑ Mitogen-induced proliferation and migration PAECs: ↑ Proliferation ↓ Apoptosis ↑ NO production and vasodilation	LY294002	Inhibits growth factor-induced PASMC proliferation and migration (159) Attenuates development of HPH in rats (17)
Akt	PASMCs PAAFs PAECs	PASMCs: ↑ Human IPAH ↑ Rat HPH PAAFs: ↑ Human IPAH ↑ Rat HPH PAECs: Unknown	PASMCs: ↑ Proliferation ↓ Apoptosis PAAFs: ↑ Proliferation PAECs: ↑ Proliferation ↓ Apoptosis ↑ NO production and vasodilation	Triciribine	Attenuates development of HPH in rats (17)

Molecule/ Pathway	Cell Type	Status in PAH/Experimental PH	Function	Inhibitor(s)/ Activator(s) Tested	Effect of Inhibitor(s)/Activator(s)
mTORC1	PASMCs	↑ Human IPAH ↑ Rat HPH ↑ Rat MCT-PH	↑ Proliferation	Rapamycin Everolimus	Inhibits proliferation of human IPAH PASMCs (97) Inhibits proliferation of rat MCT-PH PASMCs (226) Improves PVR and 6MWD in patients with severe PAH (23)
mTORC2	PASMCs	↑ Human IPAH ↑ Rat HPH ↑ Rat MCT-PH	↑ Proliferation ↓ Apoptosis	PP242 (dual)	Inhibits proliferation, induces apoptosis in human IPAH PASMCs (97) Reverses pulmonary vascular remodeling in rat HPH (97)
Notch3/HES	PASMCs	↑ Human PAH ↑ Mouse HPH	↓ Differentiation ↑ Proliferation	DAPT	Inhibits human IPAH growth (118) Reverses mouse HPH (118)
FoxOs	PASMCs	↓ Human PAH ↓ Rat MCT-PH ↓ Rat SuHx-PH	↑ Proliferation ↓ Apoptosis	Paclitaxel Abraxane	Inhibits human IPAH growth (31) Reverses rat MCT-PH (31) Reverses rat SuHx-PH (31, 190)

Definition of abbreviations: 6MWD, 6-min-walk distance; Akt, v-*akt* murine thymoma viral oncogene homolog; DAPT, *N*-[*N*-(3,5-difluorophenacetyl)-L-alanyl]-*S*-phenylglycine *t*-butyl ester; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; FGF-2, fibroblast growth factor 2; FGFR, fibroblast growth factor receptor; FoxO, forkhead-box class O; HES, hairy/enhancer of split; HPH, hypoxia-induced pulmonary hypertension; IPAH, idiopathic pulmonary arterial hypertension; MAPK, mitogen-activated protein kinase; MCT-PH, monocrotaline-induced PH; MEK, MAPK/ERK kinase; mTOR, mechanistic target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PA, pulmonary artery; PAB, pulmonary artery banding; PAAFs, pulmonary artery adventitial fibroblasts; PAECs, pulmonary artery endothelial cells; PAH, pulmonary arterial hypertension; PASMCs, pulmonary artery smooth muscle cells; PDGFR, platelet-derived growth factor receptor; PH, pulmonary hypertension; PI3K, phosphatidylinositol 3-kinase; PVR, pulmonary vascular resistance; RTK, receptor tyrosine kinase; RV, right ventricular; SuHx, Sugen/hypoxia.

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Table 3

Machine Learning Advantages, Challenges, and Pitfalls

ML Advantages	
Effective task-specific algorithms are available for a wide range of research applications	
Allows for accurate prediction and detection of complex patterns/relationships in data	
Permits agnostic and unbiased exploratory research, freedom from assumptions about underlying data	
Well-suited for high-dimensional omics data sets (where number of input variables exceeds observations)	
Able to accommodate several types of input variables (continuous, categorical, imaging features, etc.)	
Can simultaneously account for linear and nonlinear relationships between variables	
Models can autonomously improve while learning in real time from new data	
ML Challenges and Pitfalls	**Pitfall Avoidance**
Models trained on small data sets often have poor generalizability in other data sets	Collaborative data sharing and harmonization (particularly important in PAH, a rare disease)
No gold standard approaches exist for algorithm selection or hyperparameter tuning	Apply heuristic data-driven methods to objectively select algorithm and set hyperparameters
Algorithms can be oversensitive to noise (mislabeled data, confounding signal, assay technical artifact)	Careful attention to data collection, quality control, and preprocessing (normalization, standardization, missing value handling, batch adjustment)
Black box models (difficult to interpret)	Explain model decision processes (graphically); delineate which input variables drive model decisions (feature selection methods, variable importance measures)
Model decisions can unfairly disadvantage certain patient subgroups (algorithmic bias)	Select a cohort representative of wider disease population; include socioeconomic input variables
Inadequate model validation	Independent cohort validation is critical (resampling-based cross-validation on training data set is not adequate); compare model vs. existing gold standard
Lack of transparency in model reporting	Full disclosure of methods; share model code and anonymized data at publication;

Definition of abbreviations: ML, machine learning; PAH, pulmonary arterial hypertension; TRIPOD, transparent reporting of a multivariable prediction model for individual prognosis or diagnosis.

Adapted, with permission, from Rhodes CJ, et al., 2022 (152).