Update in Pulmonary Vascular Diseases and Right Ventricular Dysfunction 2019

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The American Thoracic Society journals published numerous original research studies in 2019 that advance our understanding of the molecular pathogenesis of pulmonary vascular disease, the identification of new mutations associated with the development of heritable pulmonary arterial hypertension, the role of right ventricular dysfunction in disease progression and severity, and the discovery and validation of novel imaging modalities and biomarkers that determine disease severity. These advances reflect a vital field of study and present major translational advances that serve to identify new disease targets and provide new tools to assess risk and validate surrogate endpoints for clinical trials.

Novel Molecular Mechanisms of Pulmonary Hypertension

Genetic, metabolic, and signaling abnormalities underlying pulmonary arterial hypertension (PAH) pathogenesis continue to attract significant interest, and understanding the complexity of the molecular changes driving pulmonary hypertension (PH) calls for unbiased "omics" and systems biology approaches. To facilitate discoveries of novel molecular targets, the Geraci group performed transcriptomics and a systems biology analysis of 58 PAH and 25 control lung tissues (1, 2). This largest-to-date PAH lung transcriptome study provides transcriptional framework for further mechanistic studies and will be of interest to many PH researchers. In addition to transcription, the majority of molecular events are controlled by epigenetic modifications, the most prominent of which is protein phosphorylation. The kinome profiling by the Schermuly lab identified CDKs (cyclin-dependent kinases) as most activated in pulmonary arterial vascular smooth muscle cells (PAVSMCs) and the selective CDK inhibitor palbociclib as a potent inhibitor of PAVSMC proliferation, and severe experimental PH (3), demonstrating the benefits of unbiased profiling in identifying new targets for therapeutic intervention.

Chronic hypoxia is an important factor involved in PH pathogenesis, and novel mechanisms of hypoxia-induced PH (HPH) continue to be uncovered. By performing transcriptome analysis of 32 mouse strains with HPH, Ikeda and colleagues discovered that PL/J mice develop HPH with extremely high pulmonary systolic pressures but minimal pulmonary vascular remodeling. Further analysis uncovered two possible signaling pathways contributing to this PH phenotype: mRNA-driven aberrant T-cell expression and upregulated C5a and C5AR1 (4, 5). Another study demonstrated a novel role for adhesion molecule P-selectin as a regulator of hypoxia-induced PAVSMC proliferation and PH. Fucoidan, a natural P-selectin–blocking ligand, exhibited strong antiproliferative properties and attenuated HPH in mice, providing preclinical evidence for its potential use as an antiproliferative adjuvant drug for PH associated with hypoxia (6, 7). This latter finding is particularly interesting because anti–P-selectin monoclonal antibodies have now been approved for the treatment of patients with sickle cell disease (8), allowing for future assessments of efficacy in subgroups with PAH.

Further expanding our understanding of the mechanisms driving HIV-associated pulmonary vascular pathology, Chelvanambi and colleagues reported

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an important role of HIV virally encoded protein Nef in endothelial cell (EC) apoptosis, subsequent vascular damage, and pulmonary pathology. Importantly, the authors also tested new strategies of targeting Nef-induced endothelial damage by EMAPII (endothelial monocyte–activating polypeptide II)-neutralizing antibody or PAK2 (P21-activated kinase 2) inhibitors, providing a preclinical basis for developing novel strategies to target HIV-induced pulmonary vascular disease (9, 10).

The TGFβ (transforming growth factor β)/BMP (bone morphogenetic protein) network, a key player in the PH pathogenesis, continues to attract the attention of the research community. Calvier and colleagues demonstrated that chronic exposure to TGFβ1 induces constitutive activation of canonical Smad3 signaling in PAVSMCs, supporting its role in pulmonary vascular remodeling and overall PH (11). Furthermore, interesting data from studies in PAH have now been extended to studies of the mechanism of disease of portopulmonary hypertension and the hepatopulmonary syndrome associated with advanced cirrhosis. In patients with PAH, the concentrations of BMP9 are reduced, and BMP9 has been shown to bind to BMPR2 (bone morphogenetic protein receptor type 2) for normal smooth muscle homeostatic signaling. In mouse models, the depletion of either BMPR2 or its ligand BMP9 leads to PH, and therapy with recombinant BMP9 reverses PH (12). Importantly, a recent study showed that rare variants in the gene encoding BMP9 (*GDF2*) are linked to lower BMP9 and BMP10 concentrations and are strongly associated with PAH development (13, 14). This paradigm has now been extended to portopulmonary hypertension and the hepatopulmonary syndrome, with two studies documenting low concentrations of circulating BMP9 in these conditions, which is likely related to decreased BMP9 synthesis by hepatocytes (15, 16). These findings suggest that decreased production of BMP9 by the liver can phenocopy or exacerbate reduced BMPR2 receptor expression. Supporting the importance of BMP signaling in hepatic pathophysiology, BMP9 was identified as a paracrine factor controlling sinusoidal EC homeostasis and protecting from liver fibrosis (17).

Metabolic shift toward glycolysis plays a key role in PAH pathogenesis. Adding an additional degree of complexity to the regulation of glycolysis in PAH, a new study from Novoyatleva and coauthors suggests that the activity of a known player in PAH pathogenesis, pyruvate kinase, an enzyme controlling final rate-limiting step in glycolysis, could be modulated by allosteric regulation and subsequent nuclear localization (18). Furthermore, three different studies report previously unrecognized roles for PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3) in PAH. Huo and colleagues dissected a mechanistic link between upregulated PFKFB3 and stabilization of HIF2α in pulmonary arterial endothelial cells (PAECs). PFKFB3 stimulated production of growth factors and proinflammatory mediators, consequent PAVSMC proliferation, pulmonary vascular remodeling, and PH. Importantly, molecular or pharmacological blockade of endothelial PFKFB3 prevented or delayed PH development, indicating the potential attractiveness of PFKFB3-HIF2A as a target pathway to treat PH (19). In parallel studies, Miyagawa and colleagues demonstrated the importance of PFKFB3 for maintaining EC proliferative capacity and showed its involvement in Notch1-BMPR2–driven EC–SMC interactions (20). Further supporting the importance of PFKFB3 in PAH, Su and colleagues reported that PFKFB3 in PAVSMCs elevates glycolytic lactate production, leading to upregulation of ERK1/2–calpain axis, collagen synthesis, and PAVSMC proliferation and contributing to vascular remodeling and PH (21, 22).

The role of mechanical stimulation in pulmonary vasculature is under active investigation. The most studied mechanotransducer in PAH, a YAP (yes-associated protein), is activated by vascular stiffening and promotes proliferation of pulmonary vascular cells, pulmonary vascular remodeling, and PH. Mammoto and colleagues showed that, despite its pathological role in vascular remodeling, endothelial YAP1 is required for the expression of angiogenic factor receptor Tie2, EC sprouting, and lung vascular and alveolar regeneration (23), suggesting that caution should be taken in YAP-directed therapeutic approaches to treat PAH. Expanding our understanding about the role of mechanosensing in regulating pulmonary vascular tone, Lhomme and colleagues demonstrated that mechanosensor Piezo1 promotes intrapulmonary vascular relaxation by controlling endothelial $[Ca^{2+}]_i$ (intracellular calcium) and NO production. Interestingly, in contrast with YAP, this mechanism appeared to be fully functional and likely playing a compensatory role in the development of PH (24, 25).

The PH research community pays close attention to the rigor and reproducibility of preclinical research. To improve the methodology of preclinical compound testing, the Berger, Bonnet, and Goumans laboratories performed multicenter preclinical validation of RVX208, a clinically available inhibitor of BRD4 (bromodomain-containing protein 4), an important epigenetic factor in cardiovascular diseases. Using PAECs and PAVSMCs from patients with PAH and three different rat PH models, the authors convincingly demonstrated the antiproliferative/proapoptotic, antiinflammatory, and antiremodeling benefits of RVX208 and showed that RVX208 reverses experimental PH and could be combined with the contemporary PAH standard of care. This study paves the way for a RVX208 clinical trial for patients with PAH and provides an example of rigorous preclinical testing of new therapeutics (26, 27). Of particular importance to the field is the validation and use of preclinical models that reflect human pulmonary vascular disease. One of the most robust and widely used models is the rat, which is treated with the VEGF inhibitor SU5416 (Sugen) and exposed to hypoxia. This model develops plexogenic vascular lesions, severe pulmonary vascular disease, and right ventricular (RV) hypertrophy and dysfunction. A significant controversy has played out in the pages of the *Journal* with the report that Sugen treatment also causes emphysema in the rat model (28, 29). These findings, supporting a previous study from Kasahara and colleagues (30), were debated, and a consensus was reached that studies using this model should be sure to evaluate potential changes in the lung parenchyma that could confound study outcomes. Another important advance came from Kameny and colleagues, who developed two lamb models of congenital heart disease

(CHD)-associated PH: a left pulmonary artery ligation model, which demonstrated ventricular–vascular uncoupling and adverse ventricular–ventricular interactions, and an aortopulmonary shunt placement model, which was characterized by pulmonary vascular remodeling and the presence of PAECs with apoptosis-resistant phenotype (31). These models provide a new platform for better understanding of CHD-associated PH and preclinical testing of CHD-PH–targeted therapeutics.

Advances in Inflammation and Immunity in PAH

A number of studies have highlighted the critical role of the immune system and inflammation in PAH pathogenesis. Novel genes were implicated in the complex inflammatory processes observed in PAH. TLR3 (Toll-like receptor 3), a receptor for double-stranded RNA (dsRNA) that was previously implicated in vascular protection, was found to be reduced in PAH lung tissue and ECs. Paradoxically, TLR3 deficiency was found to promote dsRNA signaling, driving greater endothelial apoptosis, pulmonary vascular remodeling, and PH progression *in vivo* (32). Another important inflammatory mediator, the danger-associated molecular pattern molecule HMGB1 (high-mobility group box-1) was found to be upregulated in the pulmonary vascular intima and perivascular adventitia; similar upregulation of TLR4, for which HMGB1 acts as a ligand, was observed (33). Importantly, neutralizing anti-HMGB1 antibody reduced pulmonary pressures and reversed pathological pulmonary vascular remodeling in the Sugen-hypoxia rat model. The investigators then specifically targeted the HMGB1–TLR4 interaction with a peptide designed to interfere with ligand-receptor binding—this treatment improved PH in both the monocrotaline and Sugen-hypoxia rat models. Such findings add to a growing body of evidence of TLR4 in PAH, which is reported to be activated by gut translocation of LPS (34).

Importantly, in addition to the implications of these findings to inflammation in general, the connection to dsRNA signaling has reinvigorated the notions of connecting various viral infections to PAH pathogenesis (35). Furthermore, by combining siRNA high-throughput screening of more than 20,000 genes with an accompanying assessment of public PAH RNA transcript data, FHIT (fragile histidine triad) was identified as a novel BMPR2 modifier in PAH (36). Given the known connections between BMPR2 signaling and inflammation, future work on FHIT may implicate a putative role for this gene in immune cell activation as well.

Beyond these genes, specific cellular contributions to PAH inflammation were also clarified. Isobe and colleagues found that endothelial-to-mesenchymal transition (EndoMT), a pathogenic shift in cellular identity increasingly appreciated in PAH, induces a CD44 variant (CD44v8–10) that binds to and stabilizes the cystine transporter subunit (xCT). These events were found to increase reduced glutathione, enhance redox defense, increase inflammatory markers, and drive pathogenic proliferation (37, 38). Interestingly, preclinical data implicated the beneficial effects of the repurposed drug sulfasalazine in PAH, most likely targeting EndoMT-derived $CD44v^{+}xCT^{hi}$ cells. Consistent with connecting EndoMT with inflammation, mesenchymal stem cell transplantation into preclinical rodent models of PAH similarly decreased multiple indices of disease, including perivascular inflammation (39).

Although focus has traditionally been centered on identifying the specific inflammatory or immune cell type involved in pulmonary vasculature dysfunction and remodeling, recent studies have advanced the notion of soluble plasma factors as driving factors in PAH. For instance, the activation of the complement cascade (alternative pathway and component 5), as induced by immunoglobulins such as IgG, was recently described as an initiating feature of human PAH (40). In response, pulmonary perivascular-specific inflammation and proliferation was found to be driven in large part by Csf2/GM-CSF. In correlation, a related complement biomarker risk panel was defined in plasma from patients with PAH. Moreover, the pathogenic importance of the soluble cytokine IL-1β in human PAH was emphasized by findings of feasibility, safety, and reduced inflammation after daily administration for 14 days of an IL-1β inhibitor, Anakinra, in a single-arm open-label Phase IB/II clinical trial of patients with PAH and RV failure (41, 42).

Studies of antiinflammatory therapies relevant to human PAH have also advanced over the past year. As mentioned earlier, a multicenter preclinical study offered complementary data implicating the beneficial and broad effects in rodent PAH of an epigenetic inhibitor of BET (bromodomain and extraterminal) proteins BRD2 and BRD4 (43). A number of these beneficial improvements centered on decreased perivascular inflammation (44), and these findings are the basis for future exploratory trials in human PAH. Another repurposed therapeutic—acetazolamide, which targets carbonic anhydrase—was found to be effective in preclinical models of PAH (45). Interestingly, the relevant mechanism was found to be mediated by a compound mechanism of macrophage carbonic anhydrase inhibition and systemic metabolic acidosis, both working in synergy to decrease inflammation (46).

Finally, in correlation with the continued development of antiinflammatory therapies, encouraging findings were reported for the use of a ^{68}Ga-mannosylated human serum albumin tracer to label inflammatory lung macrophages in positron emission tomography of rodent PAH (47). Interestingly, proof-of-concept data revealed pulmonary uptake of this tracer in specifically human patients with PAH but not in control subjects or patients with PH due to left heart disease. Such findings appear to serve as an important foundation as a noninvasive diagnostic test for PAH but also could be useful in future clinical trials of antiinflammatory therapies.

Biomarkers, Imaging, and Risk Assessment in Pulmonary Vascular Disease

The American Thoracic Society (ATS) journals featured several studies focused on novel biomarkers and imaging techniques in pulmonary vascular disease this year. In a research letter, Tanguay and colleagues demonstrated that pulmonary artery peak wall attenuation was increased in subjects with PAH compared with control subjects, and this directly correlated with increases in

pulmonary vascular resistance and survival (48). As in systemic vascular disease, chronic thromboembolic PH (CTEPH), PH associated with congenital heart disease, and Eisenmenger syndrome, pulmonary vascular calcification may provide useful prognostic information in PAH. Exercise PH continues to be an area of uncertainty and, as such, was not included in the clinical classification from the 2018 sixth World Symposium on PH (49). A small pilot study compared positron emission tomography imaging in subjects with classical PAH, subjects with exercise PH, and control subjects and found that exercise PH had distinct perfusion characteristics at rest, including greater heterogeneity than subjects with PAH and control subjects, but similar changes in perfusion distribution with exercise as control subjects (50). These preliminary findings suggest that exercise PH may be a unique, not an intermediate, phenotype. The most controversial recommendation from the World Symposium proceedings was the proposal to redefine the hemodynamic threshold for PH to a mean pulmonary artery pressure $>$20 mm Hg (49, 51). In a retrospective study of patients with hospitalized but stable heart failure with preserved ejection fraction, Nishihara and colleagues reported that mild elevations in pulmonary pressures were associated with clinical events related to heart failure and that the optimal threshold for detecting such events was a mean pulmonary artery pressure of $\geqslant$17.5 mm Hg (52). Clearly, more modest increases in pulmonary pressures identify patients at high risk of death, but how to manage and treat these patients remains an area for future investigation (51, 53–55). Together, these new studies help to shed light on unresolved controversies in the field but require confirmation.

Two studies leveraged robust datasets to explore potential PH biomarkers. A cross-sectional study of relatively healthy participants from the Framingham Heart Study characterized volumetric whole-lung computed tomography and demonstrated that cigarette smoke exposure was associated with higher average total and peripheral pulmonary vessel volumes independent of lung function (56). These findings, which are inconsistent with vascular pruning, call into question the use of radiographic imaging to detect tobacco-associated pulmonary vasculopathy. Thayer and colleagues (57) examined red cell distribution width (RDW) in an unbiased phenome-wide association study using diagnostic codes for 263 phenotypes. Elevated RDW was associated with a twofold greater risk of PAH and chronic pulmonary heart disease. These interesting epidemiologic observations generate hypotheses for future inquiry about the role of imaging-detected pulmonary vascular alterations and cross-talk between the hematologic and pulmonary vascular systems (58), respectively, but are not ready for clinical use in PH prediction and prognostication.

This year, there were a number of publications in and beyond the ATS journals that addressed risk stratification in children and adults with PAH (59–64). Current approaches to risk stratification do not include advanced RV imaging techniques or the gold standard for RV assessment, cardiac magnetic resonance imaging (MRI) (65). Lewis and colleagues (66) sought to establish cardiac MRI thresholds for risk in a cohort of 438 adult patients with PAH. RV ejection fraction was able to parse patients into low-, intermediate-, and high-risk categories, whereas RV end-systolic volume index (RVESVI) and left ventricular (LV) end-diastolic volume index identified low and high risk only. The achievement of RVESVI percentage predicted $>$227% and RV ejection fraction $>$54% was associated with a 1-year mortality of $<$5%. Furthermore, risk stratification was improved when RVESVI was incorporated into established risk assessment tools (66). In a small cohort of children with idiopathic or heritable PAH from the Netherlands, low-risk profiles—derived from 13 criteria including hemodynamics versus a noninvasive model with seven criteria—reasonably predicted survival (67). More work is needed to determine if the addition of PAH-targeted therapies directly impacts risk or if such tools outperform isolated disease metrics. A prospective longitudinal study of 221 preterm infants demonstrated that echocardiographic evidence of pulmonary vascular abnormalities at 7 days of life in combination with mechanical ventilation were associated with roughly an eightfold increase in the risk of respiratory disease in early childhood (68). This data supports the vascular hypothesis of bronchopulmonary dysplasia pathogenesis (69) and speaks to the importance of studying prenatal and perinatal events as well as life-cycle events and their impact on the pulmonary circulation.

RV Function: Mechanisms of Disease, Diagnosis and Therapeutics

In 2018, an ATS research statement identified knowledge gaps and research opportunities to advance the understanding of RV function and failure (70). Perhaps unsurprisingly, many articles published in the ATS journals in 2019 addressed methodologies for assessing RV function and investigated underlying molecular mechanisms of RV failure. Myocardial fibrosis of the RV was one area of research. Using a pulmonary artery–banding mouse model of RV dysfunction, Boehm and colleagues showed that induction of NOS2 (nitric oxide synthase 2) increased reactive oxidant formation and RV cardiac fibroblast collagen deposition (71), raising the possibility of pharmacologic NOS2 inhibition for limiting fibrotic remodeling of the RV. Crnkovic and colleagues showed that galectin-3 mediates RV fibrosis in murine and rat models of PH; however, RV function was not improved with antifibrotic therapies, and serum galectin-3 levels did not correlate with RV function in patients with PH (72). In a research letter, Jankowich and colleagues correlated RV fibrosis, as assessed by RV extracellular volume on cardiac magnetic resonance (CMR) imaging, with CMR measures of pulmonary artery stiffness in a cohort consisting predominantly of patients with World Health Organization group 2 and 3 PH (73). Similar to the work by Crnkovic, there was no correlation between RV fibrosis and measures of RV function. Taken together, these studies advance knowledge about the mechanisms of RV fibrosis but raise more questions about the impact of myocardial fibrosis on RV function (74, 75).

Several other studies also leveraged CMR imaging for assessing RV function in pulmonary vascular disease. Ruigrok and colleagues reported worse RV and LV function in patients with proximal CTEPH compared with those with distal CTEPH despite similar clinical characteristics and pulmonary vascular resistance and compliance measured with invasive hemodynamics (76). Studies published in other journals this year have used CMR

to assess RV mass and relationship to mortality in patients with PAH (77) and to quantify vena cava backflow and its relation to RV diastolic stiffness in PAH (78).

RV function was assessed in several important conditions in neonatology. In a multicenter cohort study of neonates with hypoxic ischemic encephalopathy (HIE), multiple echocardiographic measures of RV dysfunction were associated with death or brain injury, even after adjustment for LV performance and severity scores of both HIE and neonatal illness (79). Patel and colleagues showed that LV or biventricular dysfunction, but not isolated RV dysfunction, obtained within the first 48 hours of life was associated with death in neonates with congenital diaphragmatic hernia (CDH) (80). Early ventricular dysfunction was observed in almost 40% of the registry cohort; however, nearly 30% of the included infants did not have data on cardiac function. Although these results highlight the importance of LV dysfunction on outcomes in CDH, these two studies make strong cases for the early echocardiographic assessment of ventricular function in both CDH and HIE (81, 82).

Finally, two large clinical studies examined important questions about RV dysfunction in interstitial and obstructive lung disease. In a *post hoc* analysis of the INSTAGE (Efficacy and Safety of Nintedanib Co-administered with Sildenafil in Idiopathic Pulmonary Fibrosis Patients with Advanced Lung Function Impairment) trial (83), nintedanib plus sildenafil versus nintedanib alone did not improve symptoms or VC in patients with idiopathic pulmonary fibrosis (IPF) and echocardiographic RV dysfunction (84). In an analysis of computed tomography (CT) of over 3,500 ever-smokers in the COPDGene (Genetic Epidemiology of Chronic Obstructive Pulmonary Disease) Study, a decrease in pulmonary arterial blood vessel volume was associated with an increase in RV volume (85). In addition, increased RV volume was associated with worse functional capacity and mortality. Although no hemodynamic data were available in this study, these results highlight an important pulmonary vascular phenotype of patients with chronic obstructive pulmonary disease (COPD)—a subgroup of patients with COPD and RV dilation and arterial dropout who have worse symptoms and prognosis. Although PH and RV dysfunction can complicate both COPD and IPF, PAH-targeted therapies for these groups of PH are not currently indicated. How best to identify subgroups of these patients with pulmonary vascular disease—patients in whom trials of PAH-specific therapies may be warranted—remains a challenge (86, 87).

Conclusions

Advances in basic investigation continue to refine important signaling hubs that drive a vasoconstrictive, antiapoptotic, and proliferative vascular lesion that progressively restricts pulmonary blood flow and challenges the right heart (88–90). New insights into the activation of proliferative and hypoxic signaling pathways and a central role of inflammation and altered immune signaling are highlighted. The impact of RV dysfunction and its clear measurement using modern MRI and CT imaging are presented, establishing new biomarkers of disease severity and new endophenotypes of group 3 disease. We appreciate and recognize the elite work of many investigators in the pulmonary vascular disease field and welcome the steady advance of science and drive toward better therapies and outcomes for our patients. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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