Update in Pulmonary Vascular Disease 2015

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Progress in pulmonary vascular disease medicine in 2015 included important contributions across the basic, clinical, and population sciences. Key advances enhanced understanding of right heart failure pathophysiology in pulmonary arterial hypertension (PAH), particularly with respect to right ventricular (RV)–arterial coupling and biomarker profiles, and findings from seminal PAH clinical trials this year redefined patient treatment strategies for the current era. This review aims to highlight developments in these areas, as well as to summarize key discoveries from 2015 that enhance our knowledge of contemporary basic mechanisms underlying pulmonary vascular injury and, ultimately, PAH.

Advances in Pulmonary Hypertension Pathophysiology

RV–Pulmonary Arterial Coupling

Elevated morbidity and mortality in PAH is due, in large part, to right heart failure (1), which may be defined as the inability of ventricular function to meet the demands of vascular afterload. The (patho)physiology of this process may also be characterized by the relationship between RV contractility and RV afterload, which is determined by pulmonary arterial (PA) distensibility and compliance. Under pathological conditions, a decrease in the efficiency of RV pump function is observed relative to changes in RV afterload. This results in a reduction in the ratio of RV contractility (end-systolic elastance, Ees, or maximal elastance, Emax) to RV afterload (arterial elastance, Ea), which is referred to as RV–PA uncoupling (Figure 1). Although Emax generally increases in response to increasing Ea, RV contractility will ultimately decline if RV afterload is increased above a threshold level, which results in decreased Emax/Ea (1, 2).

The past year has included an exciting focus on clinical translation of coupling measurements in PAH. In particular, methods to collect data have evolved from highly specialized pressure–volume catheters to standard fluid-filled pulmonary artery catheters (3, 4) allowing assessments of RV–PA coupling clinically (5). For example, single-beat analysis can be used to measure intracardiac RV maximal isovolumic pressure directly, and, therefore, Emax; or, Emax may be estimated by assuming an intraventricular pressure of 0 at a theoretical volume of 0 (V_0) (4, 6). Both methods obviate the need for multiple measurements at varying loading conditions. Indeed, outcome data indicating that reduced RV–PA coupling (i.e., uncoupling) is associated with mortality are now available (4), and the usefulness of noninvasive methods for determining cardiac output (CO) from arterial pulse pressure wave analysis has also been reported (7).

Findings from Oliveira and colleagues corroborate prior work showing differences in normal mean pulmonary artery pressure (mPAP) and pulmonary vascular resistance (PVR) at peak exercise stratified by age, but expand on this topic by reporting that, overall, peak mPAP during exercise greater than 23 mm Hg or PVR greater than 1.2 Wood units is abnormal (8). Others have demonstrated that RV–PA uncoupling is associated with an attendant decrease in RV contractile reserve in patients with PAH during exercise (3). Incorporating CO and, thus, total pulmonary resistance (TPR) (mean pulmonary artery pressure [mPAP]/CO) into the assessment of cardiopulmonary hemodynamic responses to exercise was also suggested this year as one potential method for defining exercise-induced pulmonary hypertension. In particular, achieving a TPR greater than 3 Wood units and mPAP greater than 30 mm Hg during exercise discriminated between patients with pulmonary vascular disease and control subjects more effectively than increased mPAP alone (9). Building on this concept, an increase in the ratio of change in mPAP to change in CO between rest and exercise (mPAP–CO slope) greater than 3 mm Hg/L/min was shown to associate with further limitations in peak $\dot{V}O_2$ and ventilation in patients with interstitial lung

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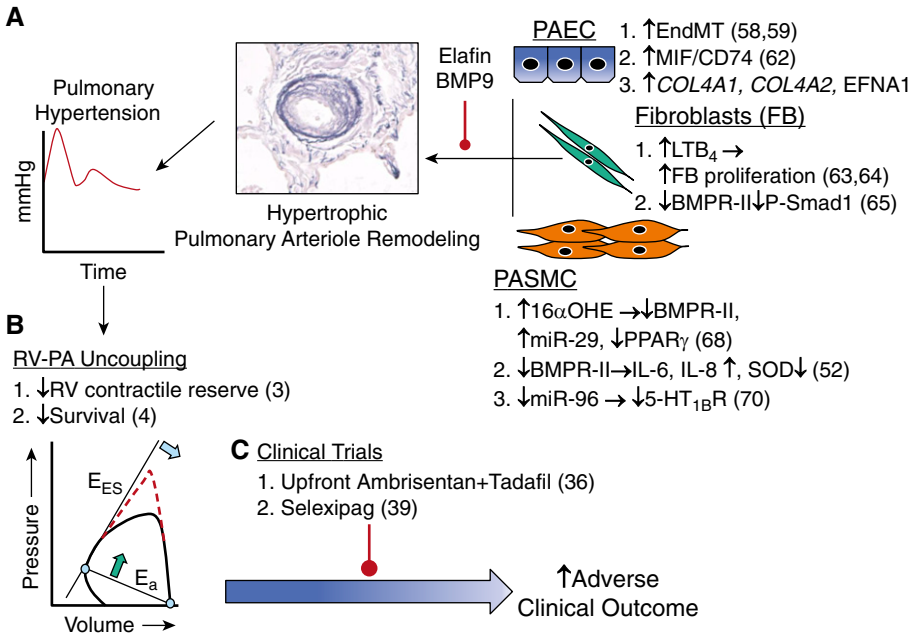

Figure 1. Integrative summary of selected findings from basic, translational, and clinical research in pulmonary vascular disease in 2015. (*A*) Vascular injury due to overactivation of inflammatory, proliferative, and fibrotic signaling pathways in pulmonary artery endothelial cells (PAECs), pulmonary artery smooth muscle cells (PASMCs), and adventitial or lung fibroblasts (FBs) induces pulmonary arteriole remodeling. (*B*) Increased pulmonary artery pressure and vascular resistance are associated with right ventricular (RV)–pulmonary arterial (PA) uncoupling. The pressure–volume diagram allows for the determination of RV end-systolic elastance (Ees). Ees is unaffected by changes to RV afterload (*dashed line*) and, therefore, is the best possible load-independent measurement of contractility. By contrast, arterial elastance (Ea) is proportional to pulmonary vascular resistance and is a measurement of RV afterload. The ratio of Ees to Ea is a measure of the coupling of the ventricular contractility to its arterial load. The ventriculovascular coupling ratio (Ees/Ea) is constant, such that ventricular contractility increases to meet increases in afterload. Thus, an increase in Ea (*dark green arrow*) in the absence of a matched increase in Ees (*light blue arrow*) as may be seen because of increases in pulmonary vascular resistance, for example, is indicative of ventriculovascular uncoupling. RV–PA uncoupling in pulmonary arterial hypertension (PAH) has been linked to impaired RV contractile reserve and increased mortality. (*C*) Two landmark clinical trials demonstrated that (*1*) upfront combination therapy with ambrisentan plus tadalafil and (*2*) treatment with the prostacyclin receptor agonist selexipag are effective strategies for improving outcome in patients with PAH. The *circle-ended line* indicates an inhibitory effect. References in text are provided parenthetically in the figure. BMP = bone morphogenetic protein; BMPR-II = bone morphogenetic protein receptor type II; *COL4A1* = gene encoding for the α-1 subunit of collagen type IV; *COL4A2* = gene encoding for the α-2 subunit of collagen type IV; EFNA1 = ephrin-A1; EndMT = endothelial–mesenchymal transition; $5\text{-HT}_{1B}R$ = 5-hydroxytryptamine receptor 1B; LTB_4 = leukotriene B_4; MIF = migration inhibitory factor; miR = microRNA; 16αOHE = 16α-hydroxyestrone; PPARγ = peroxisome proliferator-activated receptor γ; SOD = superoxide dismutase. *Panel B* and part of the figure legend are adapted by permission from Reference 71.

disease (10). Another study found that the 6-minute-walk distance (6-MWD), when combined with echocardiography, could provide a noninvasive estimate of TPR and that an mPAP–CO slope greater than 3.3 mm Hg/L/min was predictive of developing PAH (11), while alterations in the mPAP–CO slope correlate with hemodynamic improvement 1 year after pulmonary endarterectomy or after medical therapy with sildenafil for the treatment of chronic thromboembolic pulmonary hypertension (CTEPH) (12, 13).

Novel Biomarkers in PAH

Troponin-T and N-terminal pro–brain natriuretic peptide (NT-BNP) are biomarkers used commonly in clinical practice, but are surrogates of disease expression that do not provide true insights into PAH pathobiology. Markers of endothelial injury continue to be a focus of research. An initial prospective study of treatment-naive patients found elevated levels of von Willebrand factor (vWF) at baseline to be associated with worse outcome, and a more recent study of patients treated for PAH found low vWF levels to be predictive of worse outcome (14, 15). The investigators also observed an inverse association with lung transplant–free survival per 50-mg/dl reduction in total cholesterol, although a mechanism to account for this observation and findings related to vWF in PAH remains unresolved (16, 17).

In other works this year on biomarkers in pulmonary vascular disease, elevated levels of the angiostatic peptide endostatin (ES), which induces endothelial cell proliferation and migration, were observed in plasma and pulmonary arterioles from patients with PAH (18). In a derivation cohort of 84 patients with idiopathic PAH (iPAH) or connective tissue disease, plasma ES levels correlated modestly with mPAP ($r = 0.3$, $P < 0.005$) and PVR ($r = 0.3$, $P < 0.005$), while ES levels equal to or exceeding 66 ng/ml corresponded to an increase in unadjusted mortality in PAH (hazard ratio, 3.7; 95% confidence interval, 1.5–9.2; log-rank $P = 0.001$) that was directionally similar to observations from a validation cohort of patients with PAH (19). Interestingly, ES levels in this study associated with carrier status of the gene encoding ES (*COL18a1* [collagen type XVIII, α_1]); in particular, the single-nucleotide polymorphism rs12483377 was observed to cluster at a greater frequency in patients with iPAH compared with control subjects, suggesting a genetic predilection to increased ES. The potential role of collagen-associated processing enzymes as biomarkers in PAH was supported by other findings this year relating N-terminal propeptide of type III procollagen (PIIINP) to functional status, cardiac index, and 6-MWD in a similar patient cohort (20).

The importance of male or female sex as a factor in PAH prevalence, clinical outcome, and treatment response has been investigated previously (21). This year, fresh data shed novel light on the potential biomarker role of sex hormone levels in PAH. Ventetuolo and colleagues analyzed plasma levels of estradiol (E2), dehydroepiandrosterone-sulfate (DHEA-S), and testosterone in a cohort of 23 male patients with PAH and compared levels with those of 67 case-matched control subjects (22). They observed a positive association between PAH risk and E2 and E2:testosterone levels. The magnitude of the effect was dramatic for E2: the odds of PAH increased by approximately 55-fold per 1-unit increase

in the log-adjusted E2 concentration (95% confidence interval, 7.2–420.3; $P < 0.001$), and the E2 level was associated with decreased 6-MWD. By contrast, an inverse relationship between DHEA-S level and PAH risk was observed, which corresponded to a decrease in right atrial pressure and PVR on right heart catheterization.

The Biological Basis of Predicting Treatment Response in PAH

A number of efforts this year leveraged a pharmacogenomics approach to understand variable PAH treatment response patterns observed in clinical trials and in practice. In one study, a microarray analysis of immortalized circulating lymphocytes was performed in a cohort of patients with PAH stratified by a positive response to vasodilator challenge (decrease in mPAP $\geq$10 to $<$40 mm Hg with preserved CO) (23). Using a principal component analysis, the investigators identified a hierarchical organization of differentially regulated genes that could predict patient vasodilator response classification. In particular, *DSG2* and *RHOQ* informed coregulated gene clusters that accounted for the majority of variance in gene expression between the two patient groups. The authors validated this approach by predicting pulmonary vasodilator status in a separate patient cohort. Their findings were corroborated further by the fact that the two principal genes have been implicated by others in the pathobiology of PAH: *DSG2* encodes a desmosomal cadherin involved in Wnt/β-catenin signaling and pulmonary artery smooth muscle cell (PASMC) growth (24), and *RHOQ* encodes a cytoskeletal protein linked to insulin signal transduction, which, in turn, is associated with the development of vascular remodeling and pulmonary hypertension in experimental PAH *in vivo* (25).

Benza and colleagues explored a relationship between genetic variants and clinical response to treatment with the selective endothelin type A (ET_A) receptor antagonist sitaxsentan (26). They analyzed single-nucleotide polymorphisms in genes predicted to be regulated by ET-1 for 715 patients of European descent with PAH who were enrolled in the STRIDE (Sitaxsentan to Relieve Impaired Exercise) trials. In that study, a variant of the gene *GNG2* (rs11157866) was associated with a significant increase in the rate of achieving the predetermined composite endpoint of World Health Organization functional class improvement plus a sustained increase in 6-MWD compared with baseline of at least 33 m at 12 and 18 months. Furthermore, homozygous status for the minor allele T/T for rs11157866 was associated with earlier clinical benefit. Although these findings await validation from other studies, overall these data lend support to a paradigm shift emerging in pulmonary vascular medicine in which treatment selection and clinical trial candidacy may ultimately hinge on biological or genetic factors that are aligned with drug mechanism of action (27).

Novel Insights into the Pathophysiology of World Health Organization Group 2 Pulmonary Hypertension

A diastolic pressure gradient (DPG) equal to or exceeding 7 mm Hg was reported to discriminate pulmonary vascular disease in the setting of left heart dysfunction, also referred to as combined postcapillary PH and precapillary PH (28–32). DPG is the difference between pulmonary artery diastolic pressure and left ventricular (LV) end-diastolic pressure (i.e., pulmonary artery wedge pressure). However, the diagnostic and prognostic value of DPG as compared with measures such as the transpulmonary pressure gradient has been debated, suggesting a need for further studies to clarify the role of DPG in pulmonary hypertension epidemiology and outcome (28, 31, 32). To address this, Gerges and colleagues demonstrated that the prevalence of DPG equal to or exceeding 7 mm Hg is 12–14% in patients with left heart failure and, when present, is associated with more severe impairment to RV–PA coupling and less favorable outcomes (33). The prognostic variability of DPG may be related to the fact that as an indicator of PA remodeling, an elevated DPG does not necessarily imply RV dysfunction (34). In contrast, PVR and PA compliance are terms inclusive of RV function (CO and stroke volume, respectively), which have been linked consistently to outcomes in heart failure (31, 35).

PAH Treatment

Paradigm-Shifting Clinical Trial Results

Two new studies are likely to change the way patients with PAH are treated in clinical practice. The first identifies early upfront combination therapy with the ET_A antagonist, ambrisentan, and the phosphodiesterase type 5 inhibitor, tadalafil, as superior to either agent alone. The second study introduces selexipag as a new first-in-class orally delivered small-molecule prostacyclin receptor agonist.

Findings from the landmark AMBITION (Ambrisentan and Tadalafil in Patients with Pulmonary Arterial Hypertension) trial demonstrated improvements in outcome in treatment-naive patients randomized to upfront combination therapy with ambrisentan and tadalafil (36). Both drugs were titrated to full dose by 8 weeks, and at a mean follow-up of 517 days there was a 50% reduction in the primary endpoint of time to clinical worsening (i.e., death, hospitalization for worsening PAH, disease progression, or unsatisfactory long-term response) as compared with patients randomized to monotherapy with either drug. Although the treatment effect appears driven largely by differences in rates of hospitalization, it was noted that 6-MWD improved +50 m in the combination therapy arm as opposed to +24 m with monotherapy. In a separate, open-label multicenter study of 24 patients with CTD-PAH, upfront combination treatment similar to that in the AMBITION trial decreased RV mass, PVR, and PA compliance significantly at 36 weeks, as well as NT-BNP, which decreased as early as 4 weeks into therapy (37). The possibility that a specific treatment plan may influence disease trajectory has important ramifications in CTD-PAH, which is a particularly high-risk PAH subgroup characterized by impaired treatment response to conventional therapeutic strategies, as highlighted in a meta-analysis (38).

The GRIPHON (Prostacyclin [PGI2] Receptor Agonist in Pulmonary Arterial Hypertension) study randomized 1,156 patients to placebo or selexipag, which was titrated to highest tolerated dose (200–1,600 μg, twice daily). Compared with placebo, selexipag reduced by 40% the primary composite endpoint, which was driven by adjudicated disease progression (17.2% vs. 6.6%) and hospitalization (13.6% vs. 18.7%) (39). Functional improvement was modest as compared with other trials, with a 6-MWD improvement of +4 m in the treatment group as compared

with −9 m in the placebo arm at 26 weeks. The modest functional improvement in this study may be due to the high prevalence of background treatment for PAH (80%) with either an ET_A antagonist or phosphodiesterase type 5 inhibitor. Overall, GRIPHON showed an incremental benefit in clinical endpoints (primarily hospitalization and disease progression) and functional capacity beyond baseline therapy, including combination therapy with an ET antagonist or phosphodiesterase type 5 inhibitor. The improvement in clinical outcomes was relatively consistent in magnitude with that observed in AMBITION as well as the similarly clinically driven SERAPHIN (Study with an Endothelin Receptor Antagonist in Pulmonary Arterial Hypertension to Improve Clinical Outcome) trial assessing the nonselective ET receptor antagonist macitentan, noting slightly different definitions in disease progression (36, 40). Both GRIPHON and SERAPHIN had modest effects on 6-MWD, suggesting this may not be a reasonable replacement for clinical outcomes in PAH trials moving forward, particularly in studies of prevalent patients (as opposed to incident patients as studied in AMBITION).

Reconsideration of Standard Therapy

New data on existing treatment strategies for PAH have provided some much needed insights. A review of warfarin therapy in REVEAL (Registry to Evaluate Early and Long-Term PAH Disease Management) showed no survival benefit, calling into question the routine use of systemic anticoagulation in PAH (41). Similarly, the efficacy of oral treprostinil (42), which was approved by the U.S. Food and Drug Administration in 2013 based on the FREEDOM-M (Oral Treprostinil as Monotherapy for the Treatment of PAH) trial (43), was challenged by findings from a single-center study of patients enrolled in either FREEDOM-M or FREEDOM-C (Oral Treprostinil in Combination with an ERA [endothelin receptor antagonist] and/or a PDE-5I [phosphodiesterase type 5 inhibitor] for the Treatment of PAH). This study found no significant changes in 6-MWD, functional class, or hemodynamics when oral treprostinil was used at a rather low mean daily dose of 8.6 mg (44). Higher dose was correlated with lower PVR in that study, and more recent experience suggests increased dose frequency (three times daily instead of twice daily) allows for higher total daily dose with fewer dose-limiting prostanoid side effects.

New and Evolving Nonmedical Interventions for PAH

Pulmonary artery denervation and balloon angioplasty. Results at 1 year of follow-up for pulmonary artery denervation (PAD), which is a novel procedural therapy, were reported for patients with PAH (45). This was an open-label, proof-of-concept study of endovascular ablation in the left lobar artery to reduce sympathetic tone. In 66 patients with predominantly New York Heart Association (NYHA) functional class II and III symptoms, mPAP was decreased by 7 mm Hg within 24 hours of the procedure and was maintained at 1 year. The 6-MWD improved by +94 m at 1 year, and the primary outcome of PAH-related events was 15% with six PAH-related deaths. These results should be interpreted with caution given the unblinded, open-label design of the studies, and it remains to be seen whether these initially promising results can be replicated by ongoing trials (NCT 02525926, 02284737, and 02402908 at clinicaltrials.gov).

Pulmonary artery balloon angioplasty was shown to improve hemodynamics and echocardiographic markers of RV function (tricuspid annular plane systolic excursion, fractional area change, RV free wall peak strain, end-diastolic dimensions) in two small studies involving patients with inoperable CTEPH (46, 47). Angioplasty combined with pulmonary endarterectomy may also be a treatment option for selected patients (48). It should be emphasized, however, that surgical endarterectomy remains the treatment of choice and that these early unblinded studies have been conducted only in small numbers of patients with clearly inoperable disease.

Exercise therapy in PAH. Exercise training was shown to improve peak $\dot{V}O_2$ and cardiopulmonary hemodynamics at rest as well as during exercise in a prospective, randomized study of 87 patients with PAH or CTEPH (49). The exercise program started with 3 weeks of intensive therapy (1.5 h/d) at an inpatient rehabilitation facility and was then followed by a less strenuous protocol of 15 min daily, 5 days weekly at home for 12 weeks. Training was associated with a 23% increase in peak $\dot{V}O_2$ as compared with a 2% decrease in the control group. Moreover, changes in PVR from baseline by −19% and +35% were observed in the exercise and control groups, respectively. The results of this prospective, randomized study confirm key prior results, and provide clear evidence for exercise training in the care of patients with PAH (50, 51).

Pathogenesis of PAH

Bone Morphogenetic Protein Signaling

Low activity of bone morphogenetic protein receptor type II (BMPR-II) is a principal pathobiological mechanism underpinning most heritable forms of PAH, although low disease penetrance among individuals harboring the BMPR-II mutation raises speculation that acquired factors may mediate disease expression in carriers. Exaggerated inflammatory signaling is implicated as a trigger for PAH in the setting of BMPR-II functional inhibition and was studied comprehensively this year by Soon and colleagues (52). Their work demonstrated that acute treatment of PASMCs harvested from transgenic *Bmpr2*$^{+/-}$ mice with the proinflammatory cytokine LPS increased IL-6 and IL-8 levels compared with control cells. This effect was related, in part, to diminished superoxide dismutase levels, suggesting that BMPR-II may play a role in maintaining the PASMC redox balance. Indeed, LPS administration induced pulmonary hypertension in *Bmpr2*$^{+/-}$ mice *in vivo*, which was improved by administration of a nonspecific antioxidant.

Additional findings from this year lend further support to an association between BMPR-II and inflammation in the pathogenesis of CTD-PAH. For example, decreased BMPR-II-TGF-β, Smad 2/3, and Smad 1/5/8 were observed in lung samples harvested from a murine model of systemic sclerosis (i.e., TβRIIΔk-fib) and patients with systemic sclerosis (53). Abnormal BMPR-II signaling in TβRIIΔk mice was attributed to its increased proteasomal degradation, although decreased BMP sensing by BMPR-II was also observed in lung fibroblasts and corresponded to remodeling of distal pulmonary arterioles.

The possibility of BMPR-II as a modifiable treatment target in PAH was a focus of work based on the anti-elastase

effects of the peptidase inhibitor-3, elafin, which up-regulates the BMPR-II target, apelin. Elafin treatment (0.2 mg/kg) of SU-5416/Hypoxia-PAH rats restored pulmonary endothelial function and reversed distal pulmonary arteriole remodeling *in vivo*. These effects appeared to occur through increased Cav1 and endothelial nitric oxide synthase expression that induced the proliferation of normal pulmonary blood vessels (54). In converging lines of research, the administration of daily BMP9 (75 ng/d by intraperitoneal injection) reversed PAH in BMPR-II$^{R899X+/-}$ mice (which develop pulmonary hypertension spontaneously), while similar findings were observed for BMP9 treatment in other models of PAH characterized by BMPR-II underactivation, including monocrotaline-PAH and SU-5416/Hypoxia-PAH (55).

Endothelial Dysfunction

In 2015, a number of studies evaluated how endothelial dysfunction contributes to vascular disease, particularly pulmonary hypertension. In one report, the effect of erythrocyte aging during cold storage on cell deformability and hemolysis was linked to the release of cell-free hemoglobin, nitric oxide scavenging, and endothelial dysfunction in the context of red blood cell transfusions. Findings from this study demonstrated diminished endothelium-dependent blood flow in healthy individuals after transfusion with maximally aged erythrocytes (56). Impaired forearm blood flow after transfusion with aged red blood cells was associated with increased plasma levels of cell-free hemoglobin and arginase-1, which are released from hemolyzing red blood cells and inhibit nitric oxide signaling. While Risbano and colleagues evaluated the effects of transfusion of aged stored blood on endothelial function in the systemic circulation, prior studies have shown that hemolysis of aged stored blood also increases pulmonary artery pressure (57). These collective findings suggest that consideration of the age of stored blood for transfusion may be important in a range of populations for which transfusion is common, particularly patients with heart failure from LV dysfunction or pulmonary hypertension.

The importance of endothelial cell transdifferentiation also emerged this year as an important but previously underrecognized mechanism in the pathogenesis of PAH. Ranchoux and colleagues (58) studied a novel transgenic experimental mouse model of PAH (BMPR2$^{\Delta 140Ex1/+}$) and found increased endothelial migration and mesenchymal transition in pulmonary arteriole intimal and plexogenic lesions. This was characterized by up-regulation of endothelial VE-cadherin, CD31, and α-smooth muscle actin, as well as decreased expression of p120-catenin (that maintains cell–cell junction integrity). In addition, a threefold increase in pulmonary vascular levels of the endothelial–mesenchymal transition markers Twist-1 and P-vim in BMPR2$^{\Delta 140Ex1/+}$ mice was observed compared with wild-type controls, which was consistent with findings from others this year in systemic sclerosis–associated PAH lung specimens (59).

Rhodes and colleagues (60) used next-generation RNA sequencing methods to probe the expression profile of pulmonary endothelial gene sets from the lungs of patients with iPAH compared with healthy control subjects. Although decreased *BMPR2* gene expression could be anticipated, the investigators used additional models to predict unanticipated functional relationship(s) between BMPR-II and other differentially expressed genes in the data set. These included *COL4A1* and *COL4A2* that encode collagen IV; and *ephrinA1* that encodes the EFNA1 receptor, which regulates collagen IV release from endothelial cells. To validate these findings, a series of experiments was performed in EFNA knockout mice, which compared with controls demonstrated less severe hemodynamic evidence of pulmonary hypertension after injection with SU-5416 and chronic exposure to hypoxia.

Inflammation

This year, an important series of discoveries strengthened the link between inflammation and pulmonary vascular injury including cell proliferation/thickening in PAH (61). An increase in levels of the proinflammatory cytokine macrophage migration inhibitory factor (MIF) was observed in the serum of patients with PAH as compared with control subjects, which occurred, in part, through a mechanism involving T-cell lymphocytes (62). Indeed, the major histocompatibility complex class II molecule and MIF receptor, CD74, emerged as highly expressed on the surface of pulmonary artery endothelial cells (PAECs) from patients with PAH. Increased expression of the MIF–CD74 complex, in turn, was observed to promote adherence of inflammatory intermediaries linked to vascular injury, including intercellular adhesion molecule-1, vascular adhesion molecule-1, and E-selectin. These findings provide key evidence in favor of an inflammatory milieu in endothelial cells (and RV cardiomyocytes) that mediates vascular injury and plays a critical role in the development of PAH.

The importance of inflammatory signaling involving cell types other than PAECs or PASMCs to the pathogenesis of vascular remodeling in PAH was an additional scientific focus of work this year. Qian and colleagues (63) observed an important effect of macrophage-derived leukotriene B_4 (LTB_4) on pulmonary artery adventitial fibroblast proliferation, migration, and differentiation, which occurred through the cognate LTB_4 G-protein–coupled receptor BLT1 in athymic SU-5416-PAH rats. The adverse effects of LTB_4, including fibroblast proliferation, occurred via activation of p38 MAPK (mitogen-activated protein kinase). Pharmacological antagonism of LTB_4 with bestatin abrogated the adverse effects of LTB_4–p38 MAPK signaling on fibroblast proliferation, cardiopulmonary hemodynamics, and/or mortality in this experimental model of autoimmune PAH (63). In separate but converging lines of work, p38 MAPK inhibition with the compound SB203580 reversed adverse vascular remodeling and pulmonary hypertension in chronic hypoxia- and monocrotaline-PAH *in vivo* (64).

Others reported that nerve growth factor functions as a key intermediary between inflammation and pulmonary vascular cell proliferation induced by chronic hypoxia and proinflammatory toxins such as monocrotaline (65). Schistosomiasis-associated PAH is perhaps among the most definitive examples of an inflammatory pulmonary vascular disease, particularly owing to a robust $CD4^+$ T helper type 2 response triggered by the *Schistosoma* eggs. The vascular inflammatory response in this setting is mediated through cross-talk between IL-1, IL-4, IL-10, and IL-13 signaling pathways, which was supported by novel findings demonstrating that genetically engineered knockout mice for

IL-4 and IL-13 are protected from *Schistosoma*-induced pulmonary hypertension (66).

MicroRNA Biology in PAH

Understanding microRNA (miR)-dependent regulation of complex pathways for deciphering arteriole-remodeling patterns in PAH evolved further in 2015 (67). Chen and colleagues (68) reported on human lung tissue miR profiling, finding that miR-29 expression is increased approximately eightfold in BMPR-II mutant mice given the estrogen metabolite 16α-hydroxyestrone. The association of this finding with diminished peroxisome proliferator-activated receptor γ demonstrated that cell metabolism, particularly insulin resistance, is an important end-pathophenotype influenced by selected sex hormone derivative(s). In turn, treatment with an miR-29 antagomir increased lung tissue mitochondrial size and improved markers of dysregulated cell metabolism as well as insulin resistance in PASMCs and PAECs induced from pluripotent stem cells harvested from a patient with *BMPR-II*-PAH.

In related work, miR-96 emerged as an important molecular link between female sex, BMPR-II, and the 5-hydroxytryptamine receptor (5-HT_{1B}R), which has been implicated previously in the development of concentric hypertrophic pulmonary vascular remodeling and thrombosis (69). Wallace and colleagues demonstrated that repression of miR-96 in PAMSCs is observed in BMPR-II$^{R899X+/-}$ female mice and female patients with PAH, which corresponds to an in increase in serotonin and 5-HT_{1B}R–dependent cell proliferation (70).

Conclusions

These observations defined 2015 as an important year, with progress in the understanding of hemodynamics, biomarkers, treatment, and pathogenesis of PAH. Ventriculovascular (RV–PA) coupling as well as novel biomarkers such as endostatin and N-terminal propeptide of type III procollagen were linked to clinical and functional outcomes. Treatment advances included randomized clinical trial data in support of upfront combination therapy with tadalafil and ambrisentan, as well as use of the oral prostacyclin receptor agonist selexipag to improve outcome in PAH. New insights into basic mechanisms of disease, particularly with respect to BMPR-II, vascular inflammation, and sex hormone signaling, are anticipated to identify novel treatment targets in the future for patients afflicted by PAH and other diseases defined by severe pulmonary vascular remodeling. ■

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