normal PAP is considered to be 14.0 ± 3.3 mm Hg, at the 6th World Symposium on Pulmonary Hypertension held in Nice in 2018, a change in the definition of PAH was proposed that would decrease the lower limit of mPAP to 20 mm Hg and a pulmonary vascular resistance > 3 WU; however, no conclusion was made regarding whether patients with an mPAP between 20 and 25 mm Hg should be treated (11).

The ability to obtain an early diagnosis in PAH remains an important goal to be pursued. Sawada and colleagues have contributed to this quest by evaluating the performance of a large ECG-based screening program in healthy schoolchildren. Unfortunately, the studied screening program did not resolve this problem, and thus early diagnosis remains an important goal to pursue in children with PAH. ■

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

Rolf M. F. Berger, M.D., Ph.D.
University Medical Center Groningen
University of Groningen
Groningen, the Netherlands

Maurice Beghetti, M.D.
Centre Universitaire Romand de Cardiologie et Chirurgie Cardiaque Pédiatrique
University of Geneva
Geneva, Switzerland
and
Centre Universitaire Romand de Cardiologie et Chirurgie Cardiaque Pédiatrique
University of Lausanne
Lausanne, Switzerland

References

1. Berger RM, Beghetti M, Humpl T, Raskob GE, Ivy DD, Jing ZC, *et al*. Clinical features of paediatric pulmonary hypertension: a registry study. *Lancet* 2012;379:537–546.
2. Ivy DD, Abman SH, Barst RJ, Berger RM, Bonnet D, Fleming TR, *et al*. Pediatric pulmonary hypertension. *J Am Coll Cardiol* 2013;62(Suppl): D117–D126.
3. Moledina S, Hislop AA, Foster H, Schulze-Neick I, Haworth SG. Childhood idiopathic pulmonary arterial hypertension: a national cohort study. *Heart* 2010;96:1401–1406.
4. Galiè N, Rubin Lj, Hoeper M, Jansa P, Al-Hiti H, Meyer G, *et al*. Treatment of patients with mildly symptomatic pulmonary arterial hypertension with bosentan (EARLY study): a double-blind, randomised controlled trial. *Lancet* 2008;371:2093–2100.
5. Lau EMT, Humbert M, Celermajer DS. Early detection of pulmonary arterial hypertension. *Nat Rev Cardiol* 2015;12:143–155.
6. Sawada H, Mitani Y, Nakayama T, Fukushima H, Kogaki S, Igarashi T, *et al*. Detection of pediatric pulmonary arterial hypertension by school electrocardiography mass screening. *Am J Respir Crit Care Med* 2019;199:1397–1406.
7. Whitman IR, Patel VV, Soliman EZ, Bluemke DA, Praestgaard A, Jain A, *et al*. Validity of the surface electrocardiogram criteria for right ventricular hypertrophy: the MESA-RV study (multi-ethnic study of atherosclerosis-right ventricle). *J Am Coll Cardiol* 2014;63:672–681.
8. Fretz EB, Rosenberg HC. Diagnostic value of ECG patterns of right ventricular hypertrophy in children. *Can J Cardiol* 1993;9:829–832.
9. Maron BJ, Friedman RA, Kligfield P, Levine BD, Viskin S, Chaitman BR, *et al*.; American Heart Association Council on Clinical Cardiology; Advocacy Coordinating Committee; Council on Cardiovascular Disease in the Young; Council on Cardiovascular Surgery and Anesthesia; Council on Epidemiology and Prevention; Council on Functional Genomics and Translational Biology; Council on Quality of Care and Outcomes Research; American College of Cardiology. Assessment of the 12-lead electrocardiogram as a screening test for detection of cardiovascular disease in healthy general populations of young people (12-25 years of age): a scientific statement from the American Heart Association and the American College of Cardiology. *J Am Coll Cardiol* 2014;64:1479–1514.
10. van Loon RL, Roofthooft MT, Hillege HL, ten Harkel AD, van Osch-Gevers M, Delhaas T, *et al*. Pediatric pulmonary hypertension in the Netherlands: epidemiology and characterization during the period 1991 to 2005. *Circulation* 2011;124:1755–1764.
11. Simonneau G, Montani D, Celermajer DS, Denton CP, Gatzoulis MA, Krowka M, *et al*. Haemodynamic definitions and updated clinical classification of pulmonary hypertension. *Eur Respir J* 2019;53: 1801913.

Smooth Muscle Cells: A Novel Site of P-Selectin Expression with Pathophysiological and Therapeutic Relevance in Pulmonary Hypertension

Pulmonary arterial hypertension (PAH) is a debilitating disease associated with increased pulmonary artery pressures, reduced lung function and exercise ability, and progressive right heart failure (1). Endothelial dysfunction, vasoconstriction, pulmonary vascular remodeling secondary to smooth muscle cell proliferation and hypertrophy, muscularization of precapillary arterioles, and distal vessel loss are among the key pathophysiological processes in PAH (2). Present pharmacologic interventions target primarily vasoconstriction and are, as such, able to slow down, but not reverse, disease progression. Hence, therapies that could reverse the proliferative phenotype of pulmonary vascular cells and, thus, improve RV function without causing adverse effects are highly desirable (3).

P-selectin expressed on activated endothelium and platelets promotes inflammation by serving as a ligand for PSGL-1 (P-selectin glycoprotein ligand-1) on leukocytes to mediate leukocyte rolling and leukocyte–platelet aggregation, respectively

Originally Published in Press as DOI: 10.1164/rccm.201812-2242ED on December 28, 2018

(4, 5). P-selectin has been shown to be upregulated on circulating platelets and pulmonary endothelium of patients with PAH and corresponding rat models (6, 7). Although these findings suggest a role for P-selectin in promoting inflammation in PAH, a direct role for P-selectin in vascular remodeling or RV dysfunction has remained elusive. Using pulmonary vascular tissue from patients with PAH and the mouse model of chronic hypoxic pulmonary hypertension (PH), the study published in this issue of the *Journal* by Novoyatleva and colleagues (pp. 1407–1420) reports the paradigm-shifting discovery (Figure 1) that P-selectin is also expressed in pulmonary artery smooth muscle cells (PASMCs) and upregulated in PAH (8). P-selectin in PASMCs was found to be a direct transcriptional target of HIF-1α (hypoxia-induced factor-1α). Remarkably, P-selectin inhibition or genetic deletion led to reversal of pulmonary vascular remodeling and improved RV function in PH mice, highlighting the therapeutic potential of anti–P-selectin therapy. Consistent with this notion, the brown algae–derived P-selectin inhibitor fucoidan improved RV function, ameliorated inflammation, and reduced pulmonary vascular remodeling in PH mice *in vivo* and proliferation/migration of PASMCs in cell culture *in vitro* by suppressing NF-κB (nuclear factor-κB), Akt–mTOR (mammalian target of rapamycin)–p70S6K (p70 ribosomal S6 kinase), ERK (extracellular signal–regulated kinase), and p38 signaling pathways in PASMCs. Taken together, Novoyatleva and colleagues (8) establish an important role for P-selectin in the pathogenesis of PAH. In addition to P-selectin's well-established function for immune cell–cell interaction, this new role is dependent on P-selectin expression on PASMCs and P-selectin–mediated signaling driving PASMCs proliferation.

The findings of Novoyatleva and colleagues open up exciting new avenues for mechanistic speculation, experimental interrogation, and potentially clinical application. First, what is the physiological function of P-selectin in PASMCs? Immunohistochemistry, Western blot analysis, and biotin pull-down show mild expression of P-selectin in PASMCs under basal, normoxic conditions and marked upregulation and surface expression in response to hypoxia. Because of its abluminal localization, this expression does apparently not serve the classic P-selectin–mediated interaction with circulating immune cells, and so its physiological role remains elusive. It also remains to be shown whether signaling via P-selectin in PASMCs is mediated by its cognate ligand PSGL-1 expressed on infiltrating leukocytes, or whether it occurs via an endogenous ligand expressed in PASMCs or via proteoglycans or glycoconjugates in the extracellular matrix. Second, is P-selectin expression in smooth muscle cells a unique feature of the pulmonary circulation, or could it also contribute to P-selectin–mediated pathologies in the systemic circulation? For example, P-selectin–deficient mice are largely protected from the formation of atherosclerotic lesions (9), a process that is intricately linked to the dysfunction of vascular smooth muscle cells (10). Third, the mechanism by which P-selectin expressed on PASMCs promotes signaling pathways

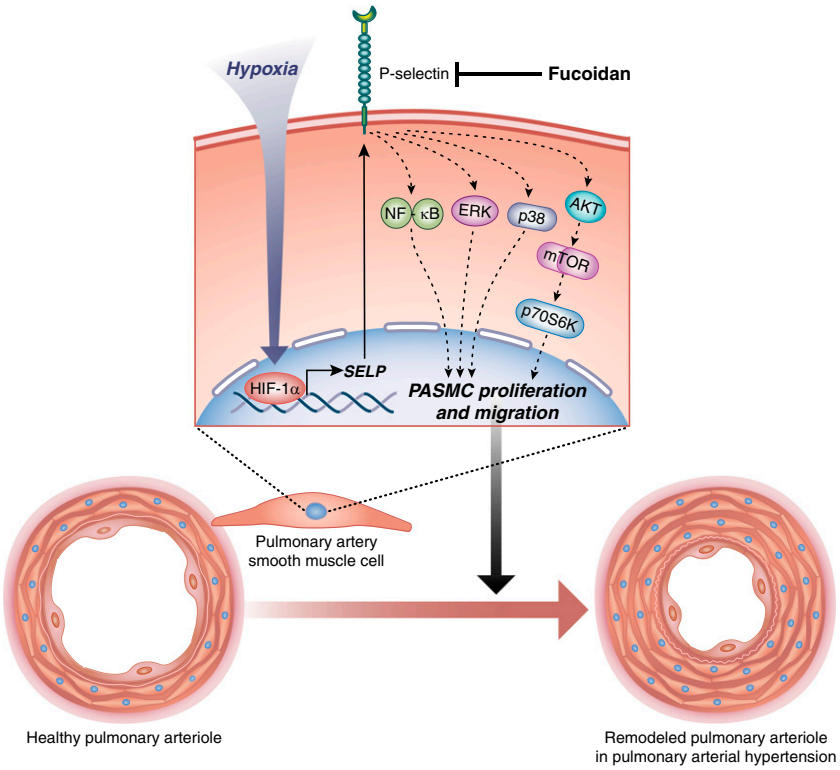

Figure 1. P-selectin in pulmonary artery smooth muscle cells (PASMCs) contributes to the pathophysiology of pulmonary hypertension. Novoyatleva and colleagues (8) show that hypoxia upregulates P-selectin in PASMCs in a HIF-1α (hypoxia-induced factor-1α)-dependent manner. P-selectin–dependent activation of NF-κB (nuclear factor-κB), Akt–mTOR (mammalian target of rapamycin)–p70S6K (p70 ribosomal S6 kinase), ERK (extracellular signal–regulated kinase), and p38 signaling pathways in PASMCs contribute to PASMC proliferation and vascular remodeling, which is reversed by the P-selectin inhibitor fucoidan. Illustration by Jacqueline Schaffer.

responsible for PASMC proliferation remains unanswered. Unlike L-selectin, P-selectin has a cytoplasmic domain that regulates its sorting into storage granules, but presumably also mediates outside-in-signaling such as P-selectin–mediated Ca^{2+} signaling in endothelial cells (11); yet how such signals relate to the stimulation of the proliferative pathways reported by Novoyatleva and colleagues remains to be elucidated. Fourth, P-selectin expression on the cell surface is commonly regulated by vesicular trafficking. Endothelial cells and platelets store P-selectin in Weibel-Palade bodies and α-granules, respectively, from where P-selectin is rapidly mobilized to the cell surface in a Ca^{2+}-dependent manner (12). PASMCs lack both Weibel-Palade bodies and α-granules, raising the question of whether P-selectin is equally stored in these cells in alternative vesicular compartments, or whether its surface expression is solely regulated by transcriptional activity and reinternalization. Fifth, is P-selectin blockade by, for example, fucoidan a feasible therapeutic strategy? PAH is a chronic disease, and therefore, reversal of PAH symptoms may require chronic therapy with anti–P-selectin agents. By blocking selectins, fucoidan effectively precludes selectin-mediated leukocyte-endothelial interaction in both the pulmonary and the systemic circulation (13). Chronic inhibition of P-selectin might hence compromise innate and adaptive immune responses leading to recurrent bacterial infections as seen, for example, in patients with leukocyte adhesion deficiency syndrome type II. Last but not least, the actual pathophysiological relevance of PASMC P-selectin in the context of PAH remains to be shown. Notably, all interventions tested by Novoyatleva and colleagues (fucoidan, blocking anti–P-selectin antibodies, or the use of P-selectin–deficient mice) will not only target PASMC P-selectin but, in parallel, block the classic P-selectin–mediated leukocyte-platelet-endothelium interaction. In light of the emerging relevance of both the immune and coagulation system in PH (14), it remains to be shown (e.g., by use of cell-specific knockout models) to what extent the observed protection is attributable to a direct role of PASMC P-selectin.

As so often after an important paradigm shift, we are left with an abundance of new questions. These should in no way diminish, but rather stress, the conceptual advancement by Novoyatleva and colleagues (8). The authors have identified a previously unrecognized site of P-selectin expression and a P-selectin dependent mechanism contributing to the progression of PAH. The findings that anti–P-selectin therapeutics such as fucoidan could be beneficial in reducing the PAH morbidity is particularly relevant in light of the fact that several P-selectin inhibitors are currently in clinical trials for treatment of auto-inflammatory diseases other than PAH (15, 16). The potential benefit of P-selectin inhibition demonstrated here may thus warrant the need for repurposing these inhibitors for treatment of patients with PAH. ■

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

Prithu Sundd, Ph.D.
Pittsburgh Heart, Lung, and Blood Vascular Medicine Institute
and
Division of Pulmonary, Allergy, and Critical Care Medicine
University of Pittsburgh School of Medicine
Pittsburgh, Pennsylvania

Wolfgang M. Kuebler, M.D.
Institute of Physiology
Charité-Universitätsmedizin Berlin
Berlin, Germany

Keenan Research Centre for Biomedical Science
St. Michael's Hospital
Toronto, Ontario, Canada
and
Department of Surgery
and
Department of Physiology
University of Toronto
Toronto, Ontario, Canada

ORCID ID: 0000-0001-7568-5719 (P.S.).

References

1. Pullamsetti SS, Schermuly R, Ghofrani A, Weissmann N, Grimminger F, Seeger W. Novel and emerging therapies for pulmonary hypertension. *Am J Respir Crit Care Med* 2014;189:394–400.
2. Tuder RM, Archer SL, Dorfmüller P, Erzurum SC, Guignabert C, Michelakis E, *et al*. Relevant issues in the pathology and pathobiology of pulmonary hypertension. *J Am Coll Cardiol* 2013;62(Suppl):D4–D12.
3. Duarte JD, Hanson RL, Machado RF. Pharmacologic treatments for pulmonary hypertension: exploring pharmacogenomics. *Future Cardiol* 2013;9:335–349.
4. Sundd P, Pospieszalska MK, Ley K. Neutrophil rolling at high shear: flattening, catch bond behavior, tethers and slings. *Mol Immunol* 2013;55:59–69.
5. McEver RP. Selectins: initiators of leucocyte adhesion and signalling at the vascular wall. *Cardiovasc Res* 2015;107:331–339.
6. Hironaka E, Hongo M, Sakai A, Mawatari E, Terasawa F, Okumura N, *et al*. Serotonin receptor antagonist inhibits monocrotaline-induced pulmonary hypertension and prolongs survival in rats. *Cardiovasc Res* 2003;60:692–699.
7. Yaoita N, Shirakawa R, Fukumoto Y, Sugimura K, Miyata S, Miura Y, *et al*. Platelets are highly activated in patients of chronic thromboembolic pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2014;34:2486–2494.
8. Novoyatleva T, Kojonazarov B, Owczarek A, Veeroju S, Rai N, Henneke I, *et al*. Evidence for the fucoidan/P-selectin axis as a therapeutic target in hypoxia-induced pulmonary hypertension. *Am J Respir Crit Care Med* 2019;199:1407–1420.
9. Dong ZM, Brown AA, Wagner DD. Prominent role of P-selectin in the development of advanced atherosclerosis in ApoE-deficient mice. *Circulation* 2000;101:2290–2295.
10. Krohn R, Raffetseder U, Bot I, Zernecke A, Shagdarsuren E, Liehn EA, *et al*. Y-box binding protein-1 controls CC chemokine ligand-5 (CCL5) expression in smooth muscle cells and contributes to neointima formation in atherosclerosis-prone mice. *Circulation* 2007;116:1812–1820.
11. Lorenzon P, Vecile E, Nardon E, Ferrero E, Harlan JM, Tedesco F, *et al*. Endothelial cell E- and P-selectin and vascular cell adhesion molecule-1 function as signaling receptors. *J Cell Biol* 1998;142:1381–1391.
12. Kuebler WM, Ying X, Singh B, Issekutz AC, Bhattacharya J. Pressure is proinflammatory in lung venular capillaries. *J Clin Invest* 1999;104:495–502.
13. Kuebler WM, Kuhnle GE, Groh J, Goetz AE. Contribution of selectins to leucocyte sequestration in pulmonary microvessels by intravital microscopy in rabbits. *J Physiol* 1997;501:375–386.
14. Kuebler WM, Bonnet S, Tabuchi A. Inflammation and autoimmunity in pulmonary hypertension: is there a role for endothelial adhesion molecules? (2017 Grover Conference Series). *Pulm Circ* 2018;8: 2045893218757596.
15. Ataga KI, Kutlar A, Kanter J, Liles D, Cancado R, Friedrisch J, *et al*. Crizanlizumab for the prevention of pain crises in sickle cell disease. *N Engl J Med* 2017;376:429–439.
16. Telen MJ, Wun T, McCavit TL, De Castro LM, Krishnamurti L, Lanzkron S, *et al*. Randomized phase 2 study of GMI-1070 in SCD: reduction in time to resolution of vaso-occlusive events and decreased opioid use. *Blood* 2015;125:2656–2664.