The Long Noncoding RNA LnRPT Puts the Brakes on Pulmonary Artery Smooth Muscle Cell Proliferation

Pulmonary arterial hypertension (PAH) affects between 15 and 50 individuals per million population (1, 2) with a 5-year survival rate for newly diagnosed patients of only 57% (3). Histologically, abnormal proliferation of pulmonary arteriolar intimal, medial, and adventitial cells, associated with perivascular fibrosis and inflammation, obliterates the vascular lumen, leading to increased pulmonary vascular resistance and right heart failure (4, 5). Many different molecular mechanisms have been linked to this common pathophenotype (6, 7). Although currently approved drugs for PAH target this shared phenotype by vasodilating narrowed pulmonary arterioles, identifying and inhibiting the underlying molecular machinery will be critical for reversing pulmonary vascular remodeling to improve the outcomes of patients with this disease.

Dysregulated proliferation of pulmonary artery smooth muscle cells (PASMCs) in the vessel media provides one potential target for drug development in PAH. Although numerous factors have been implicated (8), abnormal growth factor signaling—in particular, abnormal platelet-derived growth factor receptor (PDGFR) signaling—contributes prominently to disease pathogenesis. PDGF potently stimulates SMC proliferation through activation of PDGFR tyrosine kinases and activation of downstream signaling pathways mediated through Src family kinases, phosphatidylinositol 3-kinase (PI3K), phospholipase C γ, and Src-homology 2 domain-containing protein tyrosine phosphatase. Increased expression of PDGF and PDGFRs was observed in the lungs of patients with PAH and in animal models (8, 9) and the PDGFR inhibitor imatinib reversed the development of experimental pulmonary hypertension in two rat models of PAH (10). These observations, as well as a case report of clinical improvement with compassionate use of imatinib in a patient with severe PAH (11), supported a multinational, randomized, placebo-controlled clinical trial of imatinib in PAH. The IMPRES (Imatinib in Pulmonary Arterial Hypertension, a Randomized Efficacy Study) trial demonstrated improvements in exercise capacity and pulmonary vascular hemodynamics in patients treated with imatinib at the cost of increased serious adverse events and drug discontinuations (12). Thus, although PDGFR signaling seems to be a promising therapeutic target, alternative approaches will be required to leverage its therapeutic potential.

In this context, Chen and colleagues in this issue of the *Journal* (pp. 181–193) seek to identify the role of long noncoding RNA (lncRNA) in PDGFR signaling in PASMCs (13). LncRNAs are nonprotein-encoding RNA molecules longer than 200 bp. Most lncRNAs are synthesized by RNA polymerase II and many undergo posttranscriptional processing, including capping, splicing, and polyadenylation. LncRNAs play a variety of epigenetic regulatory roles in cells, including as activators or repressors of gene transcription, activators or repressors of mRNA translation, mRNA stabilizers, microRNA precursors, and microRNA sponges (14). In this work, Chen and colleagues identify LnRPT (lncRNA regulated by PDGF and TGF-β) as a potent regulator of PASMC proliferation.

To identify lncRNAs regulated by PDGF in PASMCs, the authors performed RNA sequencing using PASMCs isolated from rats and treated with PDGF-BB (30 ng/mL) for 12 h. After filtering the assembled transcripts and screening for coding potential, they identified 39 upregulated and 56 downregulated lncRNAs. Focusing on lncRNAs with the highest expression and conservation, they demonstrated that lentiviral-mediated knockdown of the lncRNA LnRPT induced a 1.6-fold increase in PASMC proliferation, whereas LnRPT overexpression decreased PASMC proliferation by 0.7-fold as assessed by 5-ethynyl-2′-deoxyuridine incorporation. PDGF-BB treatment downregulated LnRPT expression in both a time- and dose-dependent manner.

Next, the authors looked to identify the links between PDGFR signaling, LnRPT expression, and PASMC cell proliferation. Upstream, PDGF-mediated inhibition of LnRPT expression was inhibited by imatinib and by pictilisib, a PI3K antagonist. Inhibition of other PDGFR effectors—extracellular signal-regulated kinase, signal transducer and activator of transcription, and protein kinase C—had no effect on LnRPT levels. Returning to their RNA-sequencing data to identify genes that were differentially expressed in response to PDGF-BB treatment, the authors performed qRT-PCR to measure candidate genes implicated in the regulation of cell proliferation in response to LnRPT knockdown. Using this approach, they identified *notch3* and *jag1* as the two genes most upregulated by loss of LnRPT, and confirmed that LnRPT overexpression decreased expression of these proteins. In support of these findings suggesting that LnRPT regulates the Notch signaling pathway, treatment with the γ secretase inhibitor RO4929097 to suppress Notch signaling partially abrogated the proproliferative effects of LnRPT knockdown. The cell-cycle regulator *ccna2* was also upregulated by LnRPT knockdown.

In an effort to correlate these findings with disease, the authors demonstrated that LnRPT expression was decreased in human PASMCs after exposure to PDGF-BB and in rat pulmonary arteries after treatment with monocrotaline to induce experimental pulmonary hypertension. Although these data suggest a role for the PDGF-PI3K-LnRPT-Notch3 signaling axis in the pathobiology of PAH, additional work remains to clarify its relevance. Future efforts should focus on LnRPT expression in human PAH tissues and mechanistic studies using animal models of disease, particularly with LnRPT knockdown and overexpressing animals. Although enhanced Notch signaling correlates with PAH disease severity (15), its regulation by PDGF signaling in vascular SMCs is unclear. Notably, the authors mention that PDGF-BB treatment actually decreased expression of Notch3 in their PASMCs, which seems to suggest that other functional consequences of LnRPT silencing, such as *ccna2* upregulation, may be a more important consequence of PDGF-mediated silencing of LnRPT expression.

In summary, the authors present one of the first studies to describe a putative role for lncRNAs in the pathobiology of PAH,

and identify a novel signaling axis downstream of PDGF that is important for PASMC proliferation and may be relevant in other vascular SMCs. Perhaps most importantly, they identify LnRPT as an lncRNA that slows PASMC proliferation. Although modulating LnRPT levels in humans would be challenging, this study opens a new path to identify downstream targets of LnRPT that may be more pharmacologically tractable. This would allow investigators to leverage the therapeutic potential of the PDGFR signaling pathway while minimizing the adverse effects associated with PDGFR inhibition. ■

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References

1. Lau EMT, Giannoulatou E, Celermajer DS, Humbert M. Epidemiology and treatment of pulmonary arterial hypertension. *Nat Rev Cardiol* 2017;14:603–614.
2. Shah SJ. Pulmonary hypertension. *JAMA* 2012;308:1366–1374.
3. Benza RL, Miller DP, Barst RJ, Badesch DB, Frost AE, McGoon MD. An evaluation of long-term survival from time of diagnosis in pulmonary arterial hypertension from the REVEAL registry. *Chest* 2012;142: 448–456.
4. Rabinovitch M, Guignabert C, Humbert M, Nicolls MR. Inflammation and immunity in the pathogenesis of pulmonary arterial hypertension. *Circ Res* 2014;115:165–175.
5. Tuder RM, Marecki JC, Richter A, Fijalkowska I, Flores S. Pathology of pulmonary hypertension. *Clin Chest Med* 2007;28:23–42, vii.
6. Sutendra G, Michelakis ED. The metabolic basis of pulmonary arterial hypertension. *Cell Metab* 2014;19:558–573.
7. Machado RD, Southgate L, Eichstaedt CA, Aldred MA, Austin ED, Best DH, *et al*. Pulmonary arterial hypertension: a current perspective on established and emerging molecular genetic defects. *Hum Mutat* 2015;36:1113–1127.
8. Crosswhite P, Sun Z. Molecular mechanisms of pulmonary arterial remodeling. *Mol Med* 2014;20:191–201.
9. Humbert M, Monti G, Fartoukh M, Magnan A, Brenot F, Rain B, *et al*. Platelet-derived growth factor expression in primary pulmonary hypertension: comparison of HIV seropositive and HIV seronegative patients. *Eur Respir J* 1998;11:554–559.
10. Schermuly RT, Dony E, Ghofrani HA, Pullamsetti S, Savai R, Roth M, *et al*. Reversal of experimental pulmonary hypertension by PDGF inhibition. *J Clin Invest* 2005;115:2811–2821.
11. Ghofrani HA, Seeger W, Grimminger F. Imatinib for the treatment of pulmonary arterial hypertension. *N Engl J Med* 2005;353:1412–1413.
12. Hoeper MM, Barst RJ, Bourge RC, Feldman J, Frost AE, Galié N, *et al*. Imatinib mesylate as add-on therapy for pulmonary arterial hypertension: results of the randomized IMPRES study. *Circulation* 2013;127:1128–1138.
13. Chen J, Guo J, Cui X, Dai Y, Tang Z, Qu J, *et al*. The long noncoding RNA LnRPT is regulated by PDGF-BB and modulates the proliferation of pulmonary artery smooth muscle cells. *Am J Respir Cell Mol Biol* 2017;58:181–193.
14. Bunch H. Gene regulation of mammalian long non-coding RNA. *Mol Genet Genomics* [online ahead of print] 11 Sep 2017; DOI: 10.1007/s00438-017-1370-9.
15. Li X, Zhang X, Leathers R, Makino A, Huang C, Parsa P, *et al*. Notch3 signaling promotes the development of pulmonary arterial hypertension. *Nat Med* 2009;15:1289–1297.