Long Noncoding RNAs: Emerging Regulators of Platelet-derived Growth Factor Signaling

Pulmonary hypertension (PH) is a progressive cardiopulmonary disease characterized by pathological remodeling of the pulmonary vasculature and a concomitant increase in pulmonary vascular pressure leading to right ventricular pressure overload, eventually leading to its failure. Although several therapeutic options are available to improve the quality of life for these patients, their life expectancy is still short (1). The underlying molecular mechanisms involved in the induction and progression of the disease phenotype are yet to be fully understood. Recent studies have shown that, apart from the involvement of protein-coding genes, several microRNAs and long noncoding RNAs (lncRNAs) also play a major role in disease progression (2). LncRNAs are non–protein-coding RNAs, specifically >200 nucleotides in length, that act as key signaling modulators and drivers of various molecular pathways, as evidenced by several studies (3). Functionally, they act as decoys of chromatin modifiers, as microRNA sponges, or as scaffolds in ribonucleoprotein complexes, thereby regulating signaling pathways (3). Recently, the field of RNA therapeutics has gained widespread attention with the development of mRNA vaccines against various diseases, including coronavirus disease (COVID-19) (4). Considering the pivotal role of lncRNAs in regulating various biological processes, lncRNA-based therapies represent a promising therapeutic approach, especially for diseases with poor treatment options, such as PH. Several studies have shown that lncRNAs, such as H19, TYKRIL, MEG3, MANTIS, and HOXA-AS3, play a major role in PH development and progression (5).

Platelet-derived growth factor (PDGF) is a potent mitogen for vascular smooth muscle cells that acts via the receptor tyrosine kinase PDGFRβ. In this issue of the *Journal*, Deng and colleagues (pp. 524–538) describe the role of a novel PDGF-regulated lncRNA, lncPTSR, in controlling intracellular calcium levels and thereby contributing to the vascular remodeling in PH (6). Several studies have shown that PDGF signaling is dysregulated in patients with PH, and pharmacological interventions to modify PDGF signaling have shown promising results in both preclinical and clinical trials. The precise roles of PDGF and its downstream signaling pathways are poorly understood in PH (7). Deng and colleagues screened differentially regulated lncRNAs in rat pulmonary artery smooth muscle cells (RPASMCs) upon treatment with PDGF. They found lncPTSR to be downregulated in both RPASMCs and human pulmonary artery smooth muscle cells upon PDGF treatment and during hypoxia exposure. The authors aptly used an inhibitor-based screening method to delineate the involvement of PDGF-induced downstream molecules in downregulation of lncPTSR expression. They observed that mitogen-activated protein kinase kinase– and extracellular signal-regulated kinase–mediated downstream signaling is important in PDGF-induced downregulation of lncPTSR and also that the expression of the lncPTSR had a negative feedback effect on MAPK signaling. On the basis of its locus, the authors postulated a possible *cis* effect on the PMCA4 (plasma membrane calcium transporting ATPase 4) gene upon differential regulation of lncPTSR. They indeed show that the inhibition of lncPTSR leads to downregulation of the PMCA4 gene, disrupting the intracellular calcium clearance mechanism (Figure 1). In PH, an imbalance in intracellular calcium levels increases the vascular tone and drives the vasculature toward a proproliferative and antiapoptotic phenotype (8).

To date, several techniques have been identified to target noncoding RNAs *in vivo*, including siRNAs, shRNAs, antisense oligonucleotides such as LNA GapmeRs triggering RNase H–mediated degradation of the transcript, or CRISPR/Cas9 genome editing (9–12). Small molecules such as siRNAs or antisense oligonucleotides can be applied directly or delivered via viral vectors to target lncRNAs. Viral vector–mediated gene delivery has been tested extensively in clinical trials and has been shown to be safe and to lack serious adverse effects (13). The authors of this study skillfully used the adeno-associated virus–based shRNA system to knock down lncPTSR in rats and astonishingly observed the development of a PH-like disease phenotype even under normoxic conditions (Figure 1). As a significant modulator of both PDGF and calcium signaling, lncPTSR may serve as a potential therapeutic target for treating PH. To address the clinical and translational relevance of lncPTSR in PH, the authors might have designed experiments to overexpress lncPTSR in established PH rat models and studied its significance. In addition, Deng and colleagues also mention that lncPTSR overexpression alone did not enhance PMCA4 expression, indicating that there are other hidden players whose mode of action has yet to be deciphered. Collectively, the authors' work represents another step forward in the field of lncRNAs in PH by showing that, though still in its infancy, lncRNA-targeted therapy has the potential to be a powerful tool in treating or even curing the disease.

In PH, no clinical trials targeting lncRNAs have been performed, so factors such as RNA stability, optimal efficacy, dosing, consistency, and potential off-target effects still need to be identified (5). In line with the studies of Deng and colleagues, several other PDGF/hypoxia-induced lncRNAs, such as TYKRIL, LnRPT, and H19, have been shown to be dysregulated in PH and to modulate the PDGF signaling pathway (14–16). Thus, we need more in-depth knowledge related to the lncRNAs that are regulated under specific molecular pathways in PH, the common mechanisms driving their regulation, their additive or synergistic effects, and their cross-regulation to

Supported by Deutsche Forschungsgemeinschaft SFB-1213 (Project number 268555672) projects A01, A05 (S.S.P.) and A10 (R.S.).

Originally Published in Press as DOI: 10.1165/rcmb.2022-0029ED on March 14, 2022

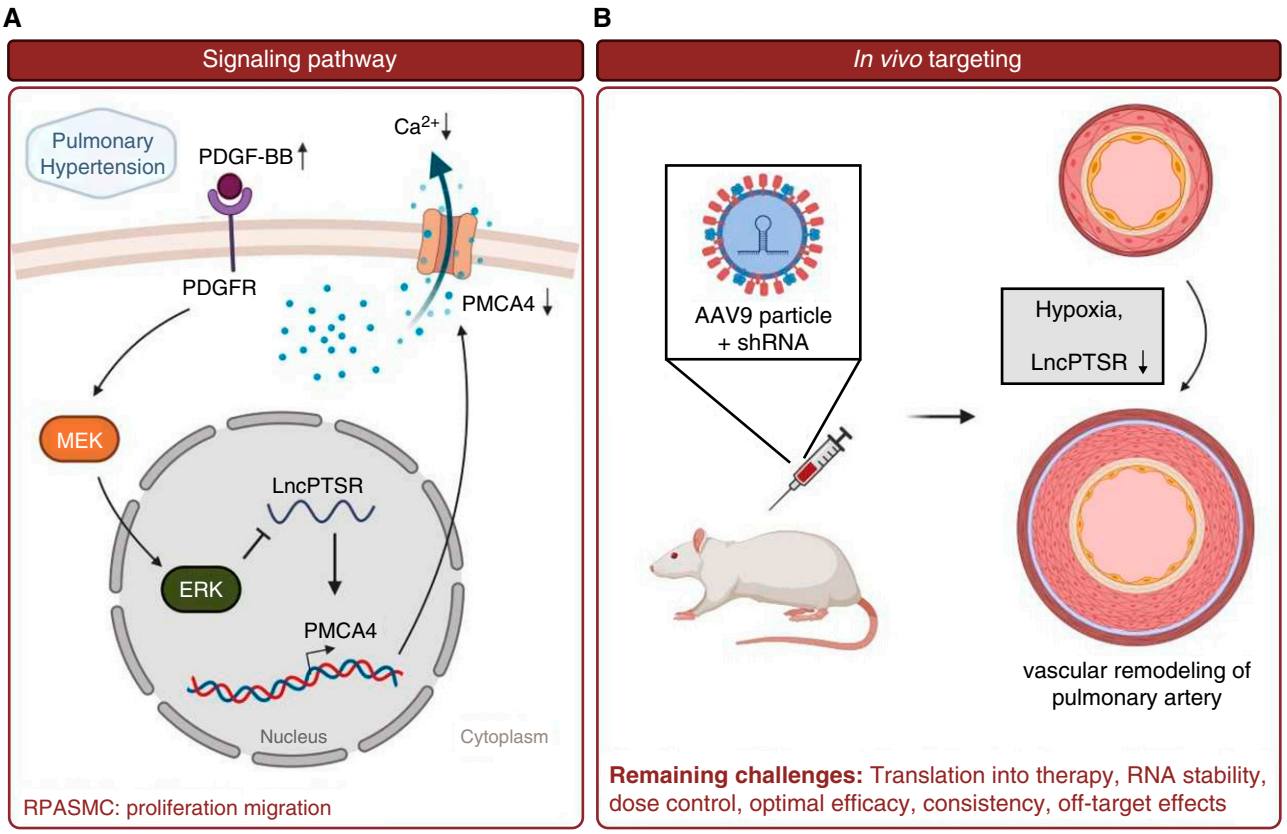

Figure 1. LncPTSR in vascular remodeling of pulmonary hypertension. (*A*) Enhanced platelet-derived growth factor (PDGF)-BB signaling in pulmonary hypertension (PH) represses expression of lncPTSR through the mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (MEK/ERK) pathway. When downregulated, PMCA4 expression is no longer enhanced through the nucleus-located lncPTSR, leading to reduced amounts of PMCA4 and increased intracellular Ca^{2+}, thereby regulating RPASMC proliferation and migration. (*B*) LncPTSR is targeted in an *in vivo* rat PH model via AAV9 particle-mediated shRNA delivery. RNA therapeutics still face several issues, such as RNA stability, dose control, efficacy, consistency, and off-target effects. AAV9 = adeno-associated virus 9; RPASMC = rat pulmonary arterial smooth muscle cells.

improve our insight and assist us in reaching our therapeutic goals. Moreover, to assure optimal lncRNA-based therapies in PH, we need to identify and target "master lncRNAs" by assessing their spatiotemporal regulation and their convergent and divergent functions in PH by employing omics and bioinformatics approaches. Although the lncRNA therapeutics field is still in its infancy, progress is being made in terms of understating the role of the lncRNA landscape in PH and its therapeutic potential. ■

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

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