control cohort. Additionally, MUC5B status and expression correlated with a UIP pattern on histology and computed tomography, suggesting its role in mediating a particular type of fibrosis independent of disease etiology.

So what can we take away from this avalanche of gene expression data? Clinically, CHP can be difficult to distinguish from IPF. The contrasting elements in this study provide more evidence that molecular classification of these difficult-to-diagnose entities will be possible and that we need to continue to move in that direction. Although "a rose by any other name might smell as sweet," for ILDs, it may be more important to understand their shared features in order for targeting therapies to have the broadest effect, while using their distinguishing features to help define them. ■

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A New "TYK" Tok Era for the Study of Long Noncoding RNAs in Pulmonary Hypertension

Pulmonary arterial hypertension (PAH) is a progressive disease characterized by increased pulmonary arterial pressure and pulmonary vascular resistance, ultimately leading to right heart failure and death. This increased vascular resistance leads to pulmonary vascular wall thickening and remodeling via phenotypic changes in proliferation and apoptosis in pulmonary arterial smooth muscle cells (PASMCs), pulmonary arterial endothelial cells (PAECs), pericytes, and fibroblasts (1). Over the past decade, appreciation has increased regarding the pervasive importance of noncoding RNA biology in controlling pulmonary vascular function and the pathogenic progression to PAH (2). Though studies of microRNAs in PAH have dominated the literature, the biologic roles of long noncoding RNAs (lncRNAs) increasingly are emerging as pathogenic hubs of disease (3).

TYKRIL and lncRNA Biology

Tens of thousands of lncRNA transcripts are encoded by the human genome. They are transcripts over 200 nucleotides long without predicted protein-coding potential. lncRNAs typically bind either proteins or other RNA molecules to enact epigenetic, transcriptional, and posttranscriptional regulation of gene expression, affecting a wide range of biological processes ranging from cell proliferation, apoptosis, and differentiation (4). lncRNAs have dynamic and specific expression patterns, are expressed in both the nucleus and cytoplasm, and are released at detectable and reproducible quantities into the circulating plasma (5). A crucial challenge in the study of these molecules is their poor sequence conservation across mammalian species, thus making analysis of their *in vivo* mechanisms of action particularly challenging.

Though a number of lncRNAs have been reported as dysregulated in tissue and plasma of subjects with PAH, the

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actions of only a few lncRNAs thus far have been implicated in pulmonary vascular pathophysiology. In this issue of the *Journal*, Zehendner and colleagues (pp. 1445–1457) screened the landscape of lncRNAs in human PAH lung tissue; they characterized the novel lncRNA TYKRIL (tyrosine kinase receptor–inducing lncRNA) in pulmonary vascular remodeling and suggest it as a new therapeutic target (6). The team began by conducting RNA-sequencing analysis of PASMCs and lung pericytes exposed to hypoxia and derived from patients with idiopathic PAH. This global screening approach allowed for the identification of numerous dynamically altered lncRNAs, including TYKRIL. In cultured PASMCs and pericytes and in lung slices from patients with PAH, the team demonstrated that TYKRIL regulates tyrosine kinase signaling by binding the tumor suppressor p53 and facilitating the transcription of platelet-derived growth factor receptor PDGFRβ, thus promoting the hyperproliferative and apoptosis-resistant phenotypes of these cells in PAH (Figure 1A).

Overall, this study offers a glimpse into the next generation of studies that are fast approaching to characterize lncRNA biology in PAH. As the first known lncRNA to regulate the central p53/PDGFRβ axis, TYKRIL may indeed serve as a key mediator across multiple cell types of PAH. Yet, because this lncRNA is not conserved in rodents, traditional approaches to study its mechanisms of action were not possible in live animals. Instead, the team used an *ex vivo* precise cut lung slice model (7), whereby explanted human lung slices containing all lung cell types could be cultured and manipulated at the molecular level. As such, the use of precision lung slices here served as a clever method to gain insight into this lncRNA's role in controlling vascular remodeling. Such a discovery platform may open up key avenues to study other nonconserved lncRNAs in human lung diseases.

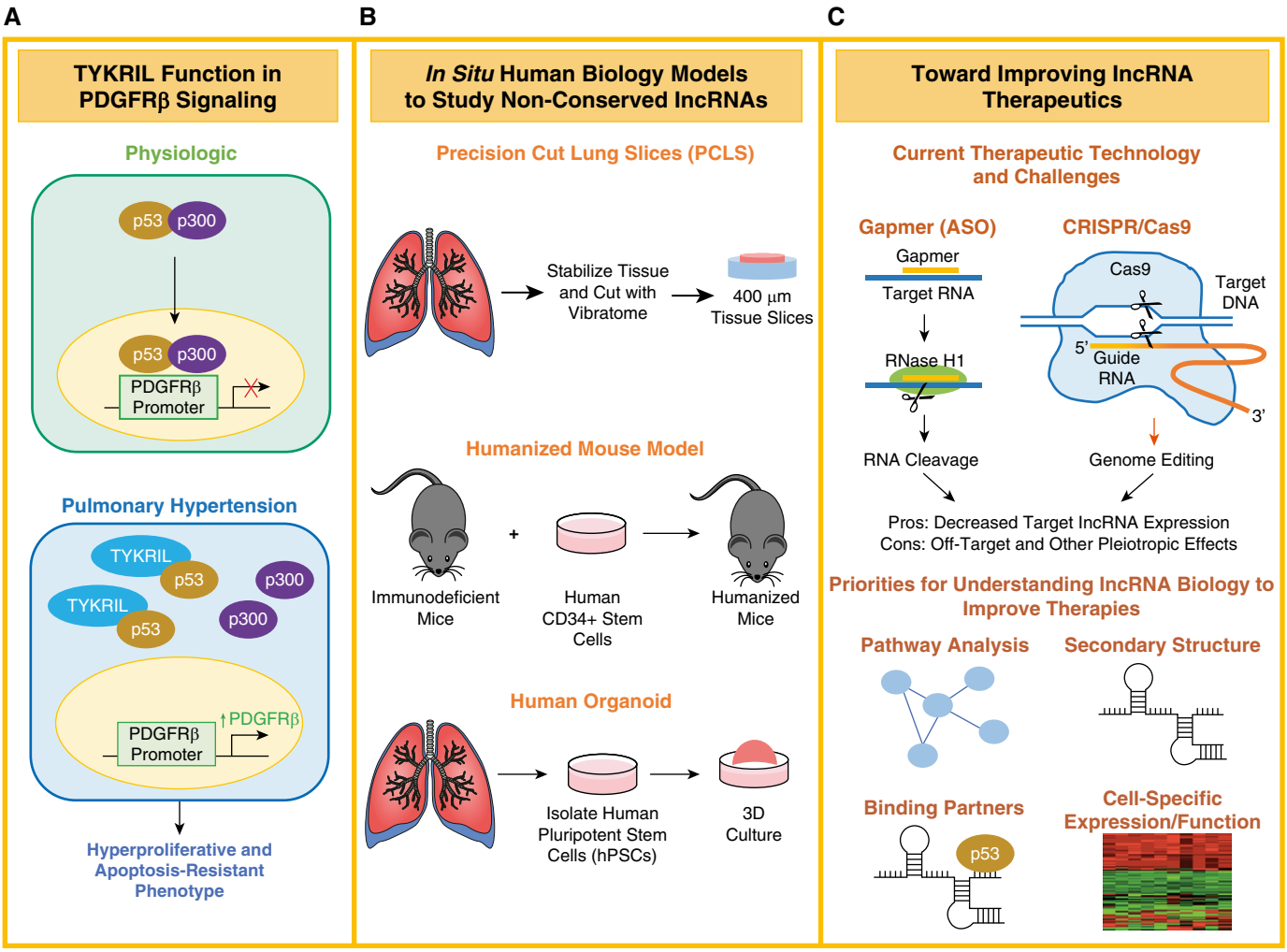

Figure 1. A new era for studies in long noncoding RNA (lncRNA) biology for pulmonary arterial hypertension. (*A*) Representation of TYKRIL (tyrosine kinase receptor–inducing lncRNA) function in the p53/PDGFRβ signaling axis under physiologic and pulmonary hypertension conditions. (*B*) Advancing methodologies for creating three *in situ* models to study human pathophysiology driven by nonconserved lncRNAs: precision-cut lung slices, humanized mouse models, and human organoid models. (*C*) Currently proposed therapeutic technologies and their drawbacks for inhibiting lncRNAs (Gapmers/antisense oligonucleotides [ASO] and CRISPR/Cas9 genome editing). Priorities for understanding lncRNA biology to develop more effective RNA therapies include systems biology analyses for defining RNA secondary structure, binding partners, and cell type–specific expression and function. 3D = three-dimensional.

Furthermore, given the well-documented limitations of current animal models of PAH (8), such work highlights the potential for future development of complex *in situ* or synthetic human biology models of PAH that otherwise have not been possible to date. For example, such approaches could be envisioned using three-dimensional human organoid modeling (9) or humanized mice engrafted with human biological tissues, often useful in modeling human immune cell interactions with the vasculature (10) (Figure 1B).

lncRNA Therapeutics

These novel discovery platforms may also open a door for development of specific lncRNAs as therapeutic targets in PAH. However, stemming from the number of unknowns that still exist in noncoding RNA biology, current technology for lncRNA inhibition may not yet be advanced enough for true therapeutic performance. Here, Zehendner and colleagues inhibit TYKRIL using gapmers, chimeric antisense oligonucleotides that engage target lncRNA and induce RNase H-based degradation (11). These can be particularly useful in targeting nuclear RNAs as compared with siRNAs that target cytoplasmic messenger transcripts. Nonetheless, gapmers and other existing RNA interference methods are difficult to implement therapeutically because of low bioavailability and off-target effects (12). Those off-target effects are further compounded by the innate biology of lncRNAs that often employs extreme and varied pleiotropic cellular reprogramming, as this group also found by RNA sequencing of cells after TYKRIL knockdown. Finally, the cell-type specificity of actions of lncRNAs such as TYKRIL can further complicate the biology. For instance, in this study, beyond pericytes and PASMCs, TYKRIL was also found to be upregulated in PAECs. However, because TYKRIL's target p53 displays divergent expression patterns and activity in PAECs in PAH (13), TYKRIL's ultimate actions may be more nuanced and distinct, depending on cell type.

Ultimately, for more reliable therapeutic development in this space, a better system would be necessary to catalog and discern the key regulatory targets and pathways of an individual lncRNA across its multilayered pleiotropy. Improvements in our ability to predict the secondary structure of lncRNAs and their binding potential to other RNAs and proteins should be prioritized. Furthermore, the development of therapeutic delivery systems *in vivo* to target or genomically edit lncRNAs in specific cells or cell types may also be warranted (Figure 1C). Despite these challenges, this study exemplifies the progress being made toward a more complete understanding and druggable landscape for lncRNAs in PAH. ■

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