JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 3, NO. 6, 2018

EDITORIAL COMMENT

TORward a Molecular Convergence Point in Pulmonary Arterial Hypertension With mTOR*

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Pulmonary arterial hypertension (PAH) is a severe cardiopulmonary disease that is characterized by an obliterative vasculopathy affecting distal pulmonary arterioles. Historically regarded as a disease of increased pulmonary arterial vasoconstriction, it is now clear that vascular remodeling in PAH is far more complex. Fibrosis, apoptosis resistance, proliferation, and a tendency toward glycolytic cellular metabolism are key endophenotypes observed in pulmonary artery endothelial cells, pulmonary artery smooth muscle cells (PASMCs), pericytes, and adventitial fibroblasts of patients with PAH (1). Even in monogenic subtypes (e.g., BMPR2 mutation), interplay between these mechanisms contributes to the wider PAH histopathophenotype, particularly plexogenic and hypertrophic vascular lesions. Realization that PAH is due ultimately to multiple overlapping molecular pathways has led to a strategic shift in the therapeutic approach clinically. Indeed, several lines of evidence suggest that targeting multiple different signaling pathways simultaneously is optimal for improving outcome in patients with PAH (2).

Despite these advances, PAH remains a morbid disease with a significant impact on longevity, quality of life, and health care expenditure (3). Arguably, there has been no major progress in the treatment of PAH for 13 years. The 14 PAH-specific drugs approved by the U.S. Food and Drug Administration modulate the same 3 key pathways, namely the endothelin-1, prostacyclin I_2, and nitic oxide pathways, and attempts at targeting novel pathways to date have experienced a high failure rate in early-phase clinical studies. One possible explanation for this failure relates to the interrelatedness, convergence, and divergence of signaling pathways that determine PAH endophenotypes (4).

The mammalian target of rapamycin (mTOR) is a protein kinase that consists of 2 functional multiprotein complexes. First, mTORC1 contains the functional subunit Raptor, is rapamycin sensitive, controls apoptosis resistance, and regulates vascular smooth muscle cell growth. Second, mTORC2 contains the functional subunit Rictor, is (generally) insensitive to rapamycin, regulates cellular adenosine triphosphate stores, and exerts effects on cellular metabolism and growth. Goncharova et al. (5) was among the first to report on the relevance of mTOR to patients with PAH and identified mTORC2-dependent activation of mTORC1 by oxidant stress as a principal cause of vascular remodeling. Subsequent studies identified mTORC1 regulation in PASMCs by hypoxia, aldosterone, and other PAH mediators (6), mTORC1 activation in pathogenic right ventricular remodeling (7), and the potential therapeutic utility of mTORC1 inhibition (with everolimus) in patients (8). Thus, a pressing need emerged to clarify the respective roles of mTORC2 and mTORC1 in PAH because each is associated with numerous important biological effects. However, definitive data on this problem have remained

*Editorials published in *JACC: Basic to Translational Science* reflect the views of the authors and do not necessarily represent the views of *JACC: Basic to Translational Science* or the American College of Cardiology.

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All authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Basic to Translational Science* author instructions page.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2018.08.003

elusive owing, in part, to complex and redundant molecular cross-talk between mTORC1 and mTORC2 and the unavailability of mTORC2-selective inhibitors.

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In this issue of *JACC: Basic to Translational Science*, Tang et al. (9) address this important issue in a report leveraging smooth muscle cell-specific conditional knockout *Raptor* (*Raptor*$^{SM-/-}$) and *Rictor* (*Rictor*$^{SM-/-}$) mice, as well as *mTOR*$^{SM-/-}$ and endothelial cell-specific conditional knockout *Rictor*$^{EC-/-}$ mice as controls. The authors provide comprehensive data using multiple methods to confirm successful cell-specific genetic modifications in vivo, and they carefully profile the histological, mechanistic, and functional consequences. The inclusion of quality control experiments verifying gene ablation and its effects is vital to initial reports introducing such disease models; indeed, the current effort is well within recent guidelines for optimizing scientific rigor in PAH (10). Although the experimental approach using bespoke transgenic models was intended to permit analyses comparing differences in mTORC1 and mTOR2 biofunctionality on vascular remodeling, the study casts a wider light on molecular hinge points that lie hidden between important pathways in PAH, with important potential translational relevance.

The central findings of the study are 4-fold (9). First, the authors observed that *Rictor*$^{SM-/-}$ mice, but not *Raptor*$^{SM-/-}$ or *mTOR*$^{SM-/-}$ mice, tended to develop mild pulmonary hypertension and pulmonary arterial remodeling spontaneously (i.e., without hypoxia stimulation). Second, *Rictor*$^{SM-/-}$ mice were protected from hypoxia-induced pulmonary hypertension, albeit to a lesser degree compared with the *mTOR*$^{SM-/-}$ or *Raptor*$^{SM-/-}$ strains. This finding confirms a contribution from both mTORC1 and mTORC2 to mTOR-mediated changes in PASMC structure and function specifically, as similar results were not observed in *Rictor*$^{EC-/-}$ mice. Third, basal (unstimulated) pulmonary arterial levels of platelet-derived growth factor receptor (PDGFR) α and β (PDGFRα and PDGFRβ) were increased significantly in *Rictor*$^{SM-/-}$ mice without an attendant increase in activated levels of the classical Rictor target, protein kinase B (Akt). This provocative observation, in turn, implies functional consequences of Rictor inhibition via alternative, Akt-independent pathways in PASMCs. Furthermore, identifying Rictor-PDGFR in vascular remodeling is particularly meaningful, as PDGFR is already implicated in the pathogenesis of PAH and a bona fide molecular target of the tyrosine kinase inhibitor imatinib. Fourth, the combination of rapamycin with imatinib was superior to monotherapy with either drug alone at attenuating right ventricular pressure assessed invasively in a hypoxia-angioproliferative model of PAH.

These exciting data (9) provide a unique perspective on mTOR biology in PAH, and, in turn, beg several potentially important additional avenues of investigations. For example, the mechanism(s) underlying spontaneous pulmonary hypertension and protection against hypoxia-induced pulmonary hypertension by Rictor inhibition remain unresolved. It is possible that nutrient bioavailability, neurohumoral regulation, mechanical stress, and other biologically dynamic determinants of mTOR activation in PAH not analyzed specifically in the study by Tang et al. (9) tilt mTORC1/2 bioactivity to modulate different phenotypes. The precise molecular mechanism by which forkhead box O3 (FOXO3A) accounts for differential mTORC1/2 bioactivity in PAH, too, was not fully investigated in this report, nor were other candidate intermediaries linking mTORC2 with PDGFR in PASMCs. It is important to address this topic further because of the promiscuity of FOXO3A, the complex relationship between Akt and Raptor/Rictor (de)activation, and the potential relevance of the Akt-FOXO3A-mTOR axis to other diseases with overlapping pathobiology as PAH and targeted by imatinib, such as solid tumor cancer (11). Also, the molecular or pathophysiologic etiology of polycythemia in the *mTOR*$^{SM-/-}$ mice was not studied in depth. Thus, analyzing further off-target effects of mTOR gene modification may glean additional insights into opportunities and limits related to modulating this pathway for therapeutic indications in the future.

Overall, these findings (9) profile in new detail the contributions of mTOR, mTORC1, and mTORC2 to pulmonary vascular remodeling and provide compelling data identifying mTORC2 as a molecular convergence point regulating 2 important, intersecting, druggable pathways: rapamycin to target mTORC1 and imatinib to target PDGFRα/PDGFRβ. Armed with this new knowledge, further study might provide an insight into the inter individual variation in clinical response to imatinib treatment. Tang et al. thus provide important mechanistic data that would support revisiting tyrosine kinase inhibition as a promising therapy (12,13). In conclusion, the current research provides a fresh view of the role

of mTOR in PASMC remodeling and offers welcomed evidence in support of an emerging paradigm shift that emphasizes interconnected pathways for optimizing basic, translational, and clinical knowledge in PAH.

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KEY WORDS cell proliferation, pulmonary hypertension, vascular biology