A Plot TWIST in Pulmonary Arterial Hypertension

The treatment of pulmonary arterial hypertension (PAH) has been a success story in pulmonary medicine. Major advances in our understanding of the mechanisms driving PAH have suggested a complicated interplay of many processes, including endothelial cell dysfunction, perivascular inflammation, smooth muscle cell hyperproliferation, and vasoconstriction (1). There are three classes of drugs that have led to improvements in symptoms and survival. Despite these advances, median survival is only 6 years (2), with the role of a potential “oncogene” in PAH would certainly fit the bill. In this issue of the Journal, Fan and colleagues (pp. 1283–1296) report their exciting findings that argue for the role of the transcription factor TWIST1 in the pathogenesis of PAH (5). How is TWIST1 relevant to PAH? TWIST1 is a well-known oncogene implicated in metastasis and resistance to chemotherapy (6). In idiopathic pulmonary fibrosis, TWIST1 transcription has been shown to be highly upregulated in idiopathic pulmonary fibrosis lungs and to promote lung fibroblast accumulation by inhibiting apoptosis (7). Similarly, in PAH, TWIST1 has already been shown to be overexpressed in the lungs and to contribute to so-called endothelial-to-mesenchymal transition through TGFβ-Smad2 signaling (8). Therefore, TWIST1 may drive this quasineoplastic pulmonary artery smooth muscle cell (PASMCE) phenotype in PAH.

In contrast to data reported in a previous study (9), Fan and colleagues have shown that TWIST1 expression is increased in PASMCEs from patients with familial PAH. Furthermore, in rodent models, PASMCE-specific loss of twist1 resulted in the attenuation of pulmonary hypertension. Overexpression of Twist1 drove PASMCE proliferation and migration and overcame the effects of harmine, a small molecule that is reported to promote TWIST1 degradation (10). To understand the mechanism behind these findings, the team turned to familiar targets, including BMP2R, the so-called PAH

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gene (11). Silencing of TWIST1 increased BMPR2 expression, and, inversely, TWIST1 overexpression decreased Bmpr2 transcription. Although this finding might suggest that TWIST1 interacts with the Bmpr2 promoter, this was not observed. Through mass spectrometry analysis the team identified a physical interaction of TWIST1 with GATA-6, a transcription factor associated with PASMC growth, and they confirmed this finding by coimmunoprecipitation. TWIST1 overexpression decreased GATA-6 protein levels despite having no effect on the level of GATA6 mRNA. This indeed was a surprising finding. TWIST1 is a transcriptional inhibitor (12). If the effect of TWIST1 on GATA-6 is not mediated by changes in mRNA levels, then does it regulate protein stability? Indeed, the authors found that reduction of GATA-6 levels driven by TWIST1 was mediated by the ubiquitin E3 ligase activity of MDM2. This reduction in GATA-6 protein levels led to decreased engagement of the BMPR2 promoter, completing the link between TWIST1 overexpression and decreased BMPR2 signaling.

This is a plot twist in our understanding of TWIST1. Instead of showing binding to the promoter regions to reduce transcription of GATA6 or Bmpr2 as might be expected of a transcription factor, the authors instead demonstrated a direct interaction between TWIST1 and GATA-6 proteins, and that this interaction led to increased proteasomal degradation of GATA-6. Although TWIST1 expression appeared to increase GATA-6–MDM2 interaction leading to GATA-6 ubiquitination, the exact mechanism by which TWIST1 promotes this interaction is not entirely clear. TWIST1 does not increase MDM2 expression, but through its interaction with GATA-6, it might induce a conformational change and increase the capacity for MDM2 binding and the destabilization of GATA-6. Further exploration of this relationship could identify a new druggable target in PAH.

Although transcription factors are notoriously difficult drug targets, the β-carboline alkaloid compound harmine has been shown to be a potent TWIST1 inhibitor (10). However, previous attempts at using harmine as a cancer therapy have been hampered by significant neurotoxicity, so the ability to create harmine derivatives with anti-TWIST1 activity and acceptable safety is an open question (13). In addition, the complete inhibition of TWIST1 may be inadvisable, as data from our group suggest that the loss of twist1 in the mesenchymal compartment may increase inflammation and worsen fibrosis (12). The effect of TWIST1 activity on the ubiquitination and degradation of GATA-6 does perhaps unveil a more promising opportunity for therapy. The ubiquitin–proteasome system has been associated with many lung diseases (14) and has been proposed as a potential therapeutic target. Ubiquitin E3 ligases and subunits, each with highly specific substrate–ligase binding pockets, are potentially amenable to small molecule inhibitors (15). The development of drugs targeting the ubiquitin system is an active area of research (16), particularly within cancer therapeutics. Notably, there are multiple ongoing cancer clinical trials examining the effects of compounds blocking the E3-ligase MDM2 (17), which the authors implicate here as being integral for TWIST1-mediated GATA-6 degradation. Perhaps a similar approach could be employed to inhibit TWIST1-driven loss of GATA-6 and reduce PASMC hypertrophy and proliferation? These exciting findings might identify a new class of therapies that may synergize with existing success stories in PAH.

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Kevin Misner, M.D., M.S.
Daniel J. Kass, M.D.
Dorothy P. and Richard P. Simmons Center for Interstitial Lung Disease and Division of Pulmonary, Allergy, and Critical Care Medicine
University of Pittsburgh
Pittsburgh, Pennsylvania

ORCID ID: 0000-0001-6597-7830 (D.J.K.).

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