

maneuver on cardiac output, pulmonary blood flow, or cerebral hemodynamics—issues of obvious relevance in future clinical trials.

One should always exercise caution when extrapolating data from animal research to the clinical setting. Indeed, the story of the journey of SI from animal research to widespread clinical use, and now perhaps a pullback after the report of sobering data from a major clinical trial, is a case in point. Nonetheless, the findings of the present study are important and clearly suggest the need for clinical investigations of different approaches to lung recruitment during stabilization of low-gestational-age neonates, with an emphasis on dynamic PEEP strategies that transiently use levels that may be well beyond the comfort level of many practitioners. ■

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## Ⓔ Involvement of PFKFB3 in Pulmonary Arterial Hypertension Pathogenesis Is It All about Glycolysis?

In mammals, cell proliferation is required for several physiological processes, including embryogenesis, growth, and proper function of several adult tissues, but it is also central to disease development, including tumorigenesis (1) and vascular remodeling (2).

Proliferating cells require nutrients, energy, and biosynthetic activity to duplicate proteins, lipids, and nucleic acids during each passage through the cell cycle (3). It is therefore not surprising that metabolic

activities in proliferating cells are fundamentally different from those in nonproliferating cells and support a platform for biosynthesis. In the 1920s, Otto Warburg described for the first time that rapidly proliferating ascites tumor cells consume glucose at a surprisingly high rate compared with normal cells and secrete most of the glucose-derived carbon as lactate rather than oxidizing it completely. The high glycolytic rate provides several advantages for proliferating cells: it allows cells to use the most abundant extracellular nutrient (i.e., glucose) to produce glucose-derived molecules necessary to biosynthetic pathways. The rate of glycolytic flux is controlled at different levels and by different mechanisms, but the first rate-limiting step is the conversion of fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F1,6P2) by 6-phosphofructo-1-kinase (PFK-1). The intracellular allosteric regulator fructose

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2,6-bisphosphate (F<sub>2,6</sub>P<sub>2</sub>) is a potent activator of PFK-1, which is synthesized and degraded by the bifunctional enzymes 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFBs) (4). The four isozymes of PFKFB (PFKFB1–4) display distinct properties, despite 85% sequence homology of their core catalytic domains. PFKFB3 especially stands out as the only inducible isoform (5) with a kinase to bisphosphatase ratio of 740:1, whereas the other isoforms are constitutive and display a more balanced ratio (6).

In this issue of the *Journal*, Kovacs and colleagues (pp. 617–627) describe for the first time the implication of PFKFB3 in pulmonary artery smooth muscle cell (PASMC) glycolysis and associated sustained proliferation, making PFKFB3 a potential new therapeutic target for the treatment of pulmonary arterial hypertension (PAH) (7). The authors found that PFKFB3 expression is up-regulated in PASMCs isolated from patients with PAH as well as in the lungs of rodent models of PAH. Smooth muscle-specific PFKFB3 knock-out mice developed attenuated hypoxia-induced pulmonary hypertension and PFKFB3 inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one decreased right ventricular systolic pressures and hypertrophy as well as pulmonary arterial remodeling by decreasing SMC proliferation. From a mechanistic point of view, Kovacs and colleagues identified an ERK1/2 (extracellular signal-regulated protein kinases 1 and 2)-mediated phosphorylation of calpain-2 at Serine 50 downstream of lactate, resulting in collagen I production and proliferation (7).

Consistent with being a transcriptional target of several oncogenic transcription factors (HIF-1 $\alpha$  [hypoxia inducible factor], Akt [serine/threonine protein kinase], PTEN [phosphatase and tensin homolog]) (8), PFKFB3 (increased expression or phosphorylation) has been involved in growth, proliferation, migration, and metastasis of various cancer cells (9). PFKFB3 inhibitor 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one and its derivative PFK15 have been shown to exhibit potent antitumor activity in several human cancer xenograft models, including tongue carcinoma, gastric cancer, and head and neck squamous cell carcinoma (9), making PFKFB3 an emerging anticancer target.

The level of glycolysis in endothelial cells is comparable to that of tumor cells and much higher than that of other healthy cells, and endothelial cells generate up to 85% of their ATP from glycolysis. A recent study demonstrated that PFKFB3-driven glycolysis is important for the migration of endothelial cells. In response to angiogenic factors, endothelial cells lacking PFKFB3 exhibit defects in the formation of filopodia and lamellipodia (10). One possible mechanism underlying this effect is that PFKFB3 compartmentalizes with F-actin in motile protrusions to provide ATP (10). Consequently, tumors implanted in mice deficient in endothelial PFKFB3 have decreased tumor blood supply and tumor size compared with control mice, demonstrating that PFKFB3 in endothelial cells is critical for tumor angiogenesis (11). In the oxygen-induced retinopathy mouse model, both the selective deletion of endothelial PFKFB3 and the application of a PFKFB3 inhibitor to mice dramatically suppressed retinal neovascularization (11). In these models, once again, lactate was identified as a critical mediator, as the addition of lactate to PFKFB3-knockdown cells rescued the suppression of endothelial proliferation and migration. The role of PFKFB3 inhibition in PAH, especially its effect on plexiform lesion and revascularization, will therefore be an important point to clarify in the near future.

Also, because hypertrophied hearts have an increased reliance on glucose with an overall reduced oxidative metabolism, it will be critical to determine the role of PFKFB3 in this cell type during right ventricular hypertrophy and failure. PFKFB3 has a variable low basal level of expression in the heart but is strongly induced by hypoxia (12), suggesting a role for this kinase during cardiac disease. Cardiac expression of a PFKFB3 kinase-deficient transgene in a transverse aortic constriction mouse model limited cardiac glycolysis and greatly exacerbated dysfunction and fibrosis (13). The results imply that stimulation of PFKFB3 and elevation of F-2,6-P<sub>2</sub> are key adaptive responses to cardiac pressure overload and might also be central to PAH-associated right ventricular hypertrophy.

Interestingly, PFKFB3 has been found to localize also in the nucleus of cancer cells, to regulate the nuclear delivery of F<sub>2,6</sub>BP and the activation of several cyclin-dependent kinases (Cdks), including Cdk-1, Cdc25C, and cyclin D3, and to decrease the expression of the cell cycle inhibitor p27 coupling by the same metabolism and proliferation (14). Among the four members of the PFKFB family, PFKFB3 is uniquely localized in the nucleus, although the reason remains unclear. PFKFB3 acetylation at lysine 472 has been shown to impair the nuclear localization signal and to accumulate PFKFB3 in the cytoplasm (15), facilitating its phosphorylation by AMPK (AMP-activated protein kinase) and enhancing glycolysis. However, because ectopic expression of nuclear PFKFB3 drives cancer cell proliferation without affecting intracellular glycolysis activity, noncanonical functions of PFKFB3 in cancer are suggested. More recently, PFKFB3 has been identified as a critical factor in homologous recombination (HR) repair of DNA double-strand breaks. PFKFB3 rapidly colocalizes with DNA damage and is critical for the recruitment of HR proteins, HR activity, and cell survival. As DNA damage repair (16) is essential for the survival of PAH-PASMCs, it remains to be established whether such a mechanism of repair involving PFKFB3 is also used by PAH-PASMCs.

The understanding of PAH energy metabolism has significantly improved in recent years, and the involvement of PFKFB3 improves it even further. Newly identified functions of the PFKFB3 enzyme that are distinct from glycolysis but still provide a potential mechanism for the coupling of metabolism and proliferation are of great interest, despite the fact that it probably increases even more the complexity of this multifactorial disease. Nevertheless, clinical trials are needed to follow up on the promising results from preclinical studies with PFKFB3 inhibitors. PFK158 is currently being tested in a dose-escalation phase 1 trial with no reported drug-related serious adverse events thus far (clinicaltrials.gov no. NCT02044861). In conclusion, the work published by Kovacs and colleagues represents an important step in our understanding of the metabolic theory of PAH and represents an attractive therapeutic avenue for PAH (7). ■

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