Hungry for Chloride: Reprogramming Endothelial Cell Metabolism in Pulmonary Arterial Hypertension

Aberrant metabolic reprogramming of pulmonary vascular cells has emerged as a major driver of pulmonary vascular dysfunction and remodeling in pulmonary arterial hypertension (PAH) (1). A major tenet in PAH relates to the shift from oxidative phosphorylation to cytoplasmic glycolysis, known as the Warburg effect (1, 2). Other hallmarks of altered metabolism in PAH include impaired glutaminolysis and fatty acid oxidation (3, 4). Most of these metabolic alterations have been described in pulmonary artery endothelial cells (PAECs) and pulmonary artery smooth muscle cells (PASMCs), even though switches to aerobic glycolysis have also been described in fibroblasts and in immune cells (5, 6).

Targeting metabolism has emerged as a potential new therapeutic paradigm, with the goal of restoring the altered balance between metabolic pathways, many of which are connected and influence each other (a phenomenon known as anaplerosis). Several trials focused on improving metabolism in PAH have been completed or are currently ongoing. For example, in a pilot trial of patients with PAH, the use of the metabolic modulator and AMPK (AMP-activated protein kinase) activator metformin was associated with improved right ventricle function and lessened right ventricle lipid accumulation (7). In another pilot trial, treatment with the pyruvate dehydrogenase kinase inhibitor dichloroacetate was associated with less severe PAH in a subset of patients with single-nucleotide polymorphisms in genes encoding for sirtuin 3 and uncoupling protein 2 (8). However, despite the increased number of clinical trials in recent years, the initiators and drivers of metabolic alterations in PAH remain incompletely understood, and new metabolic targets need to be identified. Furthermore, it remains unclear if metabolic abnormalities are universally relevant in PAH or if they are more important in some patients than others.

Chloride intracellular channels (CLICs) represent a superfamily of proteins involved in different cellular processes such as cell proliferation, apoptosis, angiogenesis, and differentiation (9, 10). Among these channels, CLICs 1 and 4 are predominantly expressed in endothelial cells (11, 12). Their expression is increased in the lung endothelium of patients with PAH, and recent papers have identified their presence in mitochondrial membranes (13, 14). CLICs 1 and 4 mediate their effects at least in part via normoxic hypoxia-inducible factor (HIF) activation (15). However, the exact mechanisms and roles of CLICs 1 and 4 in modifying mitochondrial structure and function in PAECs remain unknown.

In this issue of the *Journal*, Alzaydi and colleagues (pp. 103–115) report on their studies of the effects of CLICs 1 and 4 in PAECs from healthy donors, in blood-derived endothelial colony-forming cells (ECFCs) from patients with PAH, and a sugen/hypoxia mouse model of PAH with endothelial cell-specific deletion of CLIC 4 (16). The authors demonstrate that overexpression of CLIC proteins in healthy PAECs alters mitochondrial structure and dynamics and induces a metabolic shift toward glycolysis. Furthermore, they show that these effects are mediated through the mitochondrial fusion protein mitofusin-2. In ECFCs from patients with PAH, CLIC 1 and CLIC 4 expression were increased compared with control subjects, whereas MFN2 expression was decreased. At the metabolic level, exaggerated mitochondrial fragmentation and membrane potential, as well as cytoplasmic glycolysis, were noted in PAH, whereas cell respiration and ATP production were abrogated. Adenoviral transfer-mediated upregulation of MFN2 or silencing CLIC 1 or CLIC 4 in PAH ECFCs restored mitochondrial function and attenuated increases in glycolysis and glycolytic capacity. Lastly, endothelial-specific excision of CLIC 4 attenuated hemodynamic and structural alterations and restored the expression of mitochondrial fusion regulators in mice with sugen/hypoxia-induced pulmonary hypertension.

The study by Alzaydi and colleagues has several strengths: CLIC channels were investigated in both *in vitro* and *in vivo* models using cells from patients with PAH and transgenic mouse models and by employing elegant mechanistic loss- and gain-of-function studies. Endpoint analysis was extensive, and the scientific rigor of the reported studies was high. The results presented are consistent between the various model systems investigated. Taken together, the study by Alzayadi and colleagues robustly implicates CLICs 1 and 4, via decreased MFN2, as novel regulators of mitochondrial dysfunction and metabolic alterations in PAECs in PAH.

While innovative and intriguing, the study by Alzaydi and colleagues also raises several questions. For example, *in vivo* studies were performed in male animals only. The study of male animals to gain insights about a female-predominant disease certainly represents a limitation of the current paper, and future studies will need to identify whether the activation of CLICs 1 and 4 also plays a role in female animals (*in vitro* studies were predominantly performed in cells from female donors and patients though, suggesting that this pathway is active in females as well). Second, the mechanism(s) responsible for CLIC 1 and CLIC 4 upregulation and activation of PAH PAECs remain unknown. The authors postulate that inflammation, hypoxia, and/or genetic mutations may be responsible, but this hypothesis remains to be tested. Third, CLICs 1 and 4 are purported HIF activators. However, HIF-1 or HIF-2 inhibition did not attenuate CLIC-mediated effects, suggesting that CLICs either do not exert their metabolic effects via HIF activation or that there is compensatory upregulation of the other HIF subtype if one subtype is inhibited. This should be delineated in future studies.

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From a clinical and translational standpoint, one interesting question is whether CLICs could be targeted pharmacologically. For example, a pharmacological CLIC inhibitor (indanyloxyacetic acid-94) is available (10) and could potentially be employed in patients with PAH. However, considering the high homology among CLIC proteins (10), targeting specific CLIC channels may prove to be challenging, and off-target effects may occur. Another important concept is that of anaplerosis. It is important to consider metabolism as a unique entity because most of the metabolic pathways are interconnected. In particular, many pathways altered in PAH (such as glycolysis, fatty acid oxidation, or glutaminolysis) converge at the level of the tricarboxylic acid cycle (TCA) cycle. Thus, studying the impact of CLIC overexpression on the entire PAEC metabolome using proteomic or metabolomic approaches could determine the widespread effects of CLIC signaling on endothelial metabolism. Finally, it has recently been demonstrated that CLIC 4 inhibition in PAECs leads to increased BMPR2 (bone morphogenetic protein receptor type 2) expression (17). Considering the relevance of BMPR2 as a major regulator of PAEC homeostasis in PAH and given the strong interest in restoring impaired BMPR2 signaling in this disease, the study by Alzaydi and colleagues opens a potential new avenue for modifying BMPR2 signaling.

Alzaydi and colleagues should be congratulated for identifying CLICs as potential novel disease mediators in PAH. Restoring metabolic homeostasis clearly is one of the new frontiers in PAH, and modulating CLIC signaling may be another tool to achieve this. We are hungry for more studies in this field. ■

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