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Mitochondrial metabolism in pulmonary hypertension: beyond mountains there are mountains

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Review Series

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The Journal of Clinical Investigation

Mitochondrial metabolism in pulmonary hypertension: beyond mountains there are mountains

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Pulmonary hypertension (PH) is a heterogeneous and fatal disease of the lung vasculature, where metabolic and mitochondrial dysfunction may drive pathogenesis. Similar to the Warburg effect in cancer, a shift from mitochondrial oxidation to glycolysis occurs in diseased pulmonary vessels and the right ventricle. However, appreciation of metabolic events in PH beyond the Warburg effect is only just emerging. This Review discusses molecular, translational, and clinical concepts centered on the mitochondria and highlights promising, controversial, and challenging areas of investigation. If we can move beyond the "mountains" of obstacles in this field and elucidate these fundamental tenets of pulmonary vascular metabolism, such work has the potential to usher in much-needed diagnostic and therapeutic approaches for the mitochondrial and metabolic management of PH.

Introduction

Pulmonary hypertension (PH) is an enigmatic disease of the lung vasculature (1). Vessels are marked by endothelial dysfunction, intimal and medial proliferation and hypertrophy, vascular stiffening, in situ thrombosis, and inflammatory infiltration. Panvascular remodeling leads to progressive narrowing and occlusion of the distal arterioles (2), causing increased pulmonary vascular resistance. Mortality in pulmonary arterial hypertension (PAH) is driven by right ventricular (RV) failure, and according to the Registry to Evaluate Early and Long-Term PAH Disease Management (REVEAL Registry), the 5-year survival rates by functional classes I–IV in newly diagnosed patients are 72.2%, 71.7%, 60.0%, and 43.8%, respectively (3).

PH results from many disparate triggers and is classified clinically into five groups that determine prognosis and therapy choice (1). A particularly severe subtype, group 1 PAH, arises from idiopathic, hereditary causes, toxin exposure, and secondary disease associations, and PH in groups 2–5 is associated with myriad comorbidities (1, 4). Overlap in the molecular underpinnings of different PH types remains incompletely defined. Current treatments neither prevent nor reverse the causative pathology. Furthermore, although historically the focus was on pulmonary vessels and the RV, PH involves extrapulmonary tissues (5–7). Therefore, the development of novel treatments likely hinges on identifying molecular features that unify the multitissue dysfunction that defines PH.

Spanning over two decades, extensive basic, translational, and clinical analyses support a causative link between metabolic reprogramming and PH (8). Foundational studies demonstrated a mechanism for increased glycolysis and diminished glucose oxidation in pulmonary vascular cells and RV, paralleling Warburg's observations that proliferating tumor cells display mitochondrial respiratory repression followed by increased glucose uptake and glycolysis (9, 10). The master transcription factors HIF-1 and HIF-2α are critical effectors in this metabolic shift and are stabilized by hypoxic, inflammatory, and metabolic stress — known pathologic triggers of PH (11–13). Specifically, in PH, HIF-α–dependent upregulation of pyruvate dehydrogenase kinases 1 and 2 (PDK1/2) inhibits pyruvate dehydrogenase–mediated (PDH-mediated) conversion of pyruvate to acetyl-CoA (14, 15). The Warburg effect has been observed in both animal and human models of PH (14, 16–24). Coupled with metabolic dysregulation, mitochondrial dysfunction in PH results in evasion of mitochondrial-mediated apoptosis (14, 15, 25, 26). Mitochondrial membrane potential is specific for oxidative phosphorylation (OXPHOS) and control of protein flux across the membrane; dissipation of this potential leads to energy collapse and cytochrome *c* release, driving the intrinsic apoptotic pathway (27). Like in cancer cells, the mitochondria in PH exhibit hyperpolarized membrane potential and thus resistance to apoptosis (25, 26, 28). These metabolic perturbations, summarized in Figure 1, have reframed our understanding of PH pathogenesis (2) and are a basis for the "metabolic theory" of this disease — a concept discussed in recent reviews (29–31). While the Warburg effect has served as a conceptual anchor, the complexities of metabolic reprogramming and mitochondrial dysfunction have become the focus of intensive investigation in PH. This Review will emphasize emerging and controversial molecular concepts beyond the Warburg effect, the potential for and obstacles to clinical translation of current findings, and, finally, how metabolic dysregulation in PH may inform our clinical framework and improve disease management.

Conflict of interest: SYC has served as a consultant for Actelion, Gilead, Pfizer, and Vivus. Patent applications (SYC) have been filed regarding targeting metabolism in pulmonary hypertension (WO 2017 205595).
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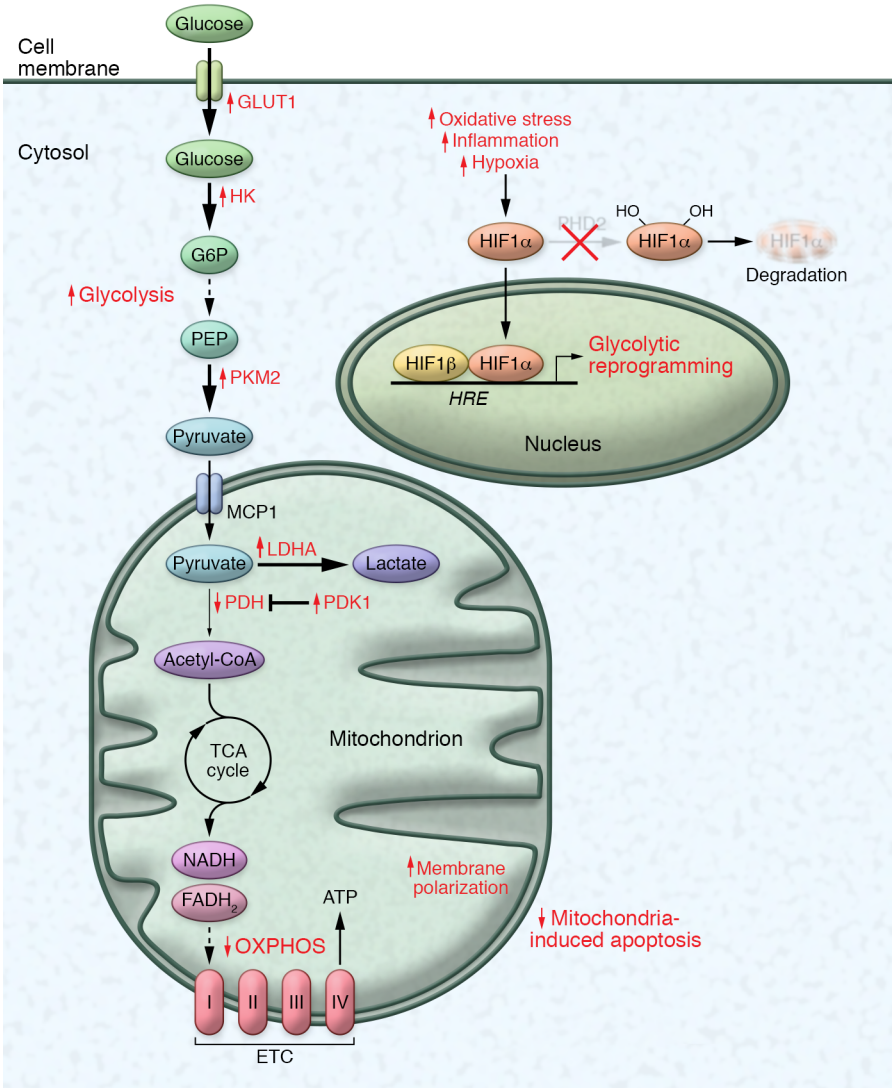

Figure 1. Metabolic pathways disrupted in PH: the Warburg effect. The mitochondria in pulmonary vascular cells and the right ventricle in PH exhibit decreased oxidative phosphorylation and increased glycolysis, consistent with the Warburg effect. Following a decrease of respiratory activity, transcriptional upregulation and stabilization of HIF-α mediates compensatory glycolytic reprogramming, which includes increased glucose transporter 1 (GLUT1); hexokinase (HK), which traps glucose via conversion to glucose-6-phosphate (G6P); and lactate dehydrogenase (LDHA), which converts pyruvate to lactic acid. At the same time, there is increased activity of pyruvate kinase isoform M2 (PKM2), which slows the production of pyruvate from phosphoenolpyruvate (PEP), and reduced pyruvate dehydrogenase (PDH) activity, driven by elevated pyruvate dehydrogenase kinase (PDK1/2). The Warburg effect promotes cell survival and proliferation, and simultaneous hyperpolarization of the mitochondrial membrane potential promotes evasion of mitochondria-dependent apoptosis. This metabolic switch in pulmonary vascular cells drives extensive remodeling that further results in increased pulmonary vascular resistance and pulmonary artery pressures. ETC, electron transport chain; HRE, hypoxia-response element.

Emerging molecular concepts

Beyond the Warburg effect

The molecular drivers of PH described in Figure 2 build on the Warburg effect, adding complexity to metabolic rewiring and highlighting a role for non-bioenergetic mitochondrial dysfunction in promoting PH. Taking cues from cancer, data demonstrate significant alterations in metabolic programs other than glycolysis and glucose oxidation, including the pentose phosphate pathway (PPP), glutaminolysis, and fatty acid (FA) synthesis and oxidation — all of which may be integral to PH initiation and progression.

Pentose phosphate pathway. While the Warburg effect focuses on glycolysis, the PPP, which yields reductive NADPH and ribose-5-phosphate for nucleotide synthesis, is often augmented in parallel with glycolysis. PPP flux is upregulated in pulmonary vascular cells in multiple PH models (16, 32–34). Increased activity of the rate-limiting enzyme glucose-6-phosphate dehydrogenase (G6PD) was observed in pulmonary artery smooth muscle cells (PASMCs) of chronically hypoxic rats (32), suggesting that G6PD deficiency may protect against PH development (35). Increased PPP flux provides defense against oxidative stress and the substrates necessary for rapid growth; therefore, G6PD may represent an enticing target for curbing PASMC hyperproliferation. However, G6PD loss also prevents production of NADPH, a reducing equivalent and critical substrate for NO synthesis (36), suggesting that G6PD inhibition might be detrimental to vasomotor tone in PH. Separately, patients with sickle cell disease, who are at increased risk of developing PH (37), have demonstrated less sickling and hemolysis with increased PPP flux compared with glycolysis. It is not yet known whether targeting aberrant PPP in PH has therapeutic effects, especially in cells heavily reliant on this pathway or exposed to oxidative stress.

Glutaminolysis. Beyond the shift in energy production ascribed to the Warburg effect, insights detail how adequate biomass is made available for vascular cell proliferation in PH. Glutaminolysis is a type of anaplerotic reaction in which tricarboxylic acid (TCA) cycle carbon intermediates are replenished, particularly when rapidly dividing cells require substantial biomass. TCA intermediates from glutaminolysis contribute to amino acid, FA, and de novo purine and pyrimidine biosynthesis (38).

New data support a prominent pathogenic role for glutamine metabolism in PH (16, 39, 40). Prior work showed that RV cardiomyocyte hypertrophy in monocrotaline-treated rats relies on

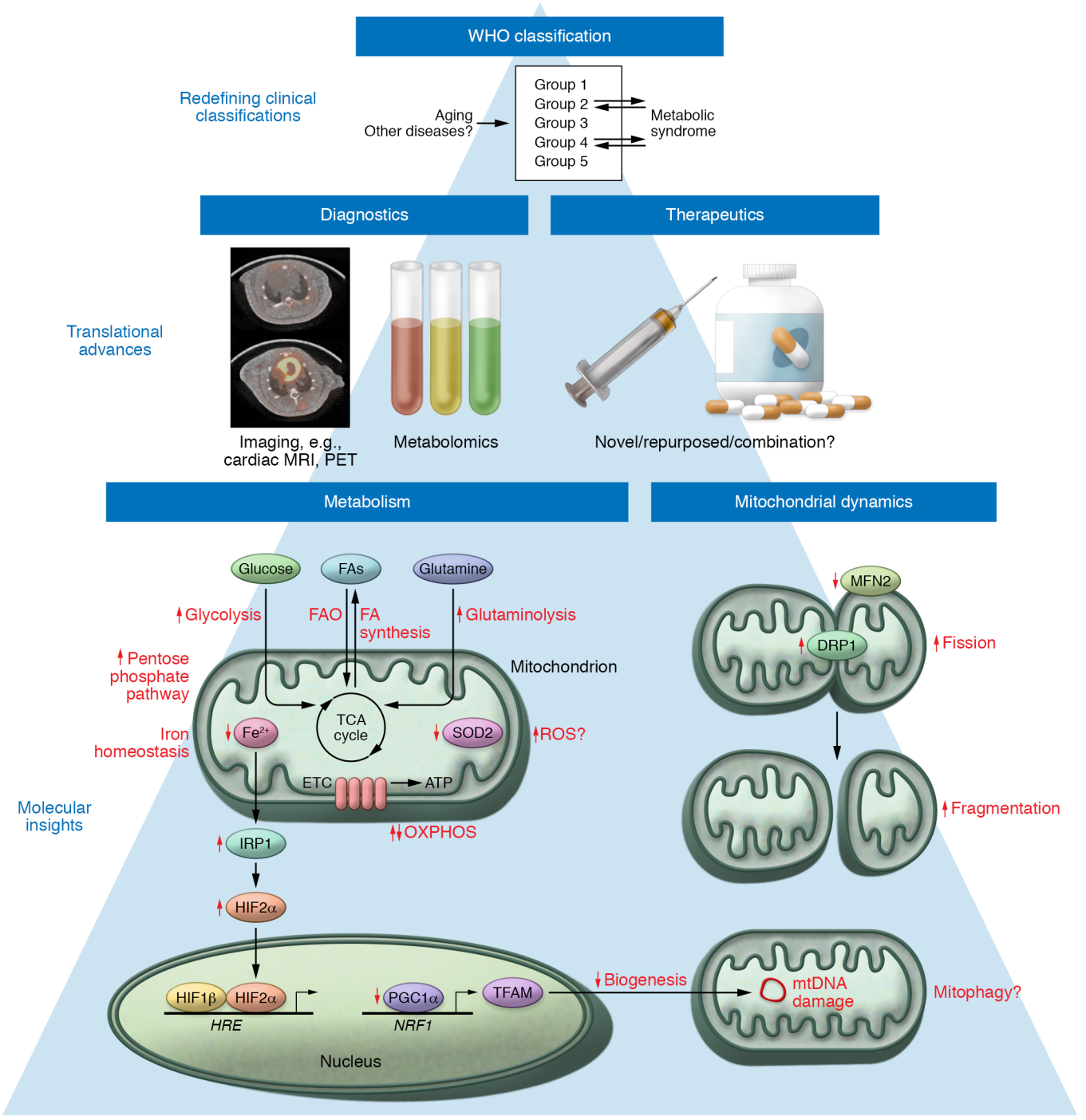

Figure 2. Emerging concepts of mitochondrial dysfunction in PH. Metabolic dysregulation in PH beyond the Warburg effect includes alterations in the pentose phosphate pathway (PPP), glutaminolysis, and FA handling; in certain contexts, it may include an increase of oxidative phosphorylation (a reverse of the Warburg effect); increased reliance on metabolic activities of HIF-2α and ROS signaling; and profound alterations of iron metabolism. Perturbations in mitochondrial dynamics involve altered mitochondrial biogenesis as well as increased fission and decreased fusion. Dysregulated mitochondrial mass and fragmentation result in metabolic reprogramming and tissue-specific dysfunction typical of PH. A more precise understanding of the complex molecular drivers of PH will inform novel diagnostic technologies and mitochondria-specific therapies. Development of imaging tools such as PET (image courtesy of J. Latoche and C. Anderson, In Vivo Imaging Facility at Hillman Cancer Center, UPMC) and cardiac MRI, high-throughput metabolomic analysis, as well as potential metabolic targeted therapies will be facilitated by a more granular understanding of mitochondrial pathology. Advancements in molecular and translational research may ultimately allow for a redefinition of PH subtypes through the lens of metabolic dysfunction, with great utility in strategizing appropriate precision medicine therapies. The processes by which other mitochondrial and metabolically driven diseases may be related to PH are yet to be determined. ETC, electron transport chain; HRE, hypoxia-response element.

glutaminolysis, which was reversed by the glutamine antagonist 6-diazo-5-oxo-L-norleucine (DON) (39). Furthermore, in pulmonary vascular cells exposed to increasing extracellular matrix stiffness, a recently identified PH trigger (41), mechanoactivation of Yes-associated protein 1 (YAP) and the transcriptional coactivator with a PDZ-binding motif (TAZ, also known as WWTR1), induced the reductive enzyme glutaminase 1 (GLS1), thus promoting glutaminolysis and hyperproliferation (40). Glutaminolytic reprogramming was increased in the vascular lesions of PAH patients and animal models, and multiple vascular cell types in PH relied on the YAP/TAZ–GLS1 axis (42–45). Pharmacologic inhibition of either YAP or GLS1 also effectively improved rodent PAH. Notably, the YAP/TAZ–GLS1 axis induced glutaminolysis in SIV-induced PAH primates as well as in HIV-infected PAH patients, suggesting a mechanistic link between glutaminolysis and this enigmatic PAH subtype. Building on these findings, a separate study demonstrated that elevated glutamate, following GLS1-mediated conversion of glutamine, stimulated NMDA-type glutamate receptors, induced glutamatergic cell-cell signaling in pulmonary vessels, and promoted PH (46). Aberrant glutamine metabolism represents an increasingly promising diagnostic and therapeutic target in PH, given its fundamental roles in promoting proliferative capacity and potentially mediating pathogenic cell-cell interactions.

FA handling. Given that FA oxidation (FAO) accounts for 60%–90% of cellular energy production in ventricular tissue (39), lipid uptake and metabolism in PH have been primarily studied in the failing RV, but with still incompletely defined results. Patients with PAH display increased circulating free FAs as well as RV-specific accumulation of increased long-chain FAs, triglycerides, and ceramides — hallmarks of lipotoxicity (47–49). Pulmonary artery banding in rats increased RV FAO (50). Conversely, RV FAO was impaired in *Bmpr2*-mutant mice, whose mutations predispose to PH (48, 49). How FAO is altered in PH is not fully defined. Notably, FAO and glucose oxidation share a reciprocal mechanism, the Randle cycle, in which activation of one inhibits the other (51). Taking advantage of this relationship, pharmacologic inhibition of FAO by trimetazidine or ranolazine increased glucose oxidation and ameliorated RV hypertrophy in animals with PH (50).

Beyond the RV, altered FA metabolism has been observed in diseased pulmonary vasculature. FA synthase, which converts malonyl-CoA to free FA, was upregulated in PASMCs and endothelial cells in PH (52, 53). Separately, genetic knockdown of malonyl-CoA decarboxylase, a critical FAO enzyme, or treatment with trimetazidine upregulated glucose oxidation in PASMCs and protected animal models from developing PH (54). These data suggest that exploiting the Randle cycle could prevent metabolic dysfunction in the pulmonary vasculature, as in the RV. However, efficacy of FAO inhibitors in PH patients depends on whether FAO-dependent metabolic dysfunction is specific to different pulmonary vascular cell types. Specifically, diminished FAO, due to decreased acetyl-CoA, promoted endothelial-mesenchymal transition (EndoMT) in multiple vascular beds, including the pulmonary vasculature, suggesting that FAO is critical for maintenance of endothelial cell fate and linking it to EndoMT and PH development (55). Separately, endothelial cells in the peripheral circulation rely heavily on FAO for nucleotide synthesis (56); whether this mechanism contributes differentially to pulmonary versus peripheral endothelium is unclear (57). Additionally, carnitine acyltransferase (CrAT), a "carnitine shuttle" component required for FA transport and metabolism, prevents acyl-CoA accumulation and promotes FAO. Decreased CrAT activity resulted in mitochondrial dysfunction and reduced FA metabolism in the endothelium of lambs with shunt-induced increased pulmonary blood flow, a model of congenital heart disease and PH (58), suggesting that targeting FAO may worsen metabolic dysfunction in this context. Thus, FA metabolic regulation in PH may be cell- and etiology-specific, potentially complicating direct therapeutic targeting.

A reverse Warburg effect in PH?

While most data support that diminished OXPHOS drives metabolic dysfunction in PH, contemporary evidence reveals that increased glucose oxidation may mediate PH pathogenesis. Specifically, methamphetamine use, which significantly increases PH risk and is associated with poor prognosis (59), prevented pulmonary endothelial adaptation to additional environmental stressors like hypoxia, thus increasing OXPHOS, mitochondrial ROS, and DNA damage (60). Separately, mitochondrial activity in platelets from PAH patients exhibited increased glycolysis and respiratory reserve capacity dependent on FAO (61). This metabolic shift in both glycolysis and respiration correlated with clinical measurements of increased mean pulmonary arterial pressure (MPAP), pulmonary vascular resistance, and RV stroke work index (61). Further study is required to determine whether such metabolic alterations in platelets alter thrombosis formation versus resolution, particularly in chronic thromboembolic PH (group 4 PH) (62). Together, these data suggest that metabolic reprogramming in PH appears to be context-specific and more complex than what is seen in hypoxic exposure alone. Thus, under certain pathogenic triggers, in specific tissues, or at certain times of disease development, the converse of the classic Warburg effect may be increasingly observed. With the advent of metabolomic and transcriptomic analyses of PH patients, a systems biology approach may facilitate our understanding of how these metabolic perturbations are interconnected and how future therapies might efficiently reverse these changes.

A central metabolic role for HIF-2α in PH

HIF-1α plays an integral role in promoting and potentiating the Warburg effect; however, its homolog HIF-2α may be equally or more important in PH, as demonstrated in patients with enhanced HIF-2α signaling. Patients with Chuvash polycythemia, a von Hippel–Lindau–associated disease characterized by HIF-2α stabilization (63), and patients with HIF-2α gain-of-function mutations develop PH (64–66). Similarly, there is increased prevalence of a gene variant representing a gain-of-function HIF-2α mutation in cattle with high-altitude PH, or brisket disease (67). Genetic manipulation in animal models of PH reinforces the importance of HIF-2α. Heterozygous HIF-2α–knockout mice were protected from hypoxic PH (68), and endothelial prolyl hydroxylase 2 (PHD2) knockdown promoted HIF-2α–dependent pulmonary vascular remodeling and PH in mice (69, 70). HIF-2α exhibits both redundant and unique functions compared with HIF-1α, and HIF-2α may be more specific to endothelial dysfunction compared with HIF-1α signaling in PASMCs (71–74). Initial mechanistic studies demonstrated that

HIF-2α upregulated arginase, a urea cycle enzyme, thus decreasing arginine availability for NO production and attenuating endothelium-dependent vasodilation (74, 75). Furthermore, HIF-2α modulated mitochondrial superoxide dismutase 2 (SOD2) and thus ROS production in other diseases (76). SOD2 epigenetic silencing has been linked to PH (77), but whether this controls a robust pathophenotype in PH is unknown. While additional studies are required to elucidate the unique effects of HIF-2α versus HIF-1α or even versus HIF-3α (78) on mitochondria in PH, current evidence suggests that there may be therapeutic utility in HIF-2α–targeting strategies in the pulmonary vasculature.

Complex alterations in mitochondrial ROS

Imbalance in multiple types of ROS is causatively linked to PH development and is reviewed extensively elsewhere (79). The predominant forms of mitochondrial ROS, superoxide, and hydrogen peroxide (H_2O_2) are of particular interest in PH because of their role in mitochondrial DNA (mtDNA) damage, cellular signaling, inflammation, and survival (79–81).

The mechanisms of mitochondrial ROS production are not fully defined and are even controversial. In general, a quorum of studies has suggested that the vasculature of nonhypoxic PAH models exhibits decreased mitochondrial H_2O_2 (14, 15). Specifically, reduced H_2O_2 production due to epigenetic silencing of SOD2 in PASMCs (77) resulted in decreases redox potential, HIF-1α stabilization, inhibition of redox-sensitive voltage-gated Kv1.5 channels, and increased intracellular calcium, which promoted proliferation (14). In models of persistent PH of the newborn (PPHN) and chronic hypoxia, decreased endothelial SOD (82) and extracellular SOD (83), respectively, further suggest that decreased H_2O_2 production may be a common feature in PH. Conversely, separate studies using a chronic hypoxia model of PH demonstrated increased mitochondrial superoxide production followed by upregulated HIF-1α and metabolic reprogramming in PASMCs (84–86). The mechanistic discrepancies in mitochondrial ROS production may depend on experimental conditions, species differences, and perhaps complexities of how pure hypoxic stress may interface with other triggers of PAH. Development of more sensitive, specific, and stable ROS probes and modulatory reagents, particularly in vivo, is needed to understand the temporal (87), subcellular (84), and tissue-, etiology-, and species-specific alterations of mitochondrial ROS in PH.

Bioactive iron deficiency in PH: focus on mitochondria

Mitochondrial iron metabolism encompasses three major pathways: iron storage, iron-sulfur (Fe-S) cluster formation, and heme synthesis (88). Moreover, an association of iron deficiency with PH has emerged (89). Because iron is required for PHD2 activity and thus HIF-α degradation, iron deficiency promoted enhanced HIF-α signaling in a rat model of PH (90, 91); similarly, deficiency of ascorbate, another cofactor required for PHD2 activity, was associated with PH (92). Iron regulatory protein 1 (IRP1) enhances translation of proteins in iron metabolism, including HIF-2α, and has been implicated in PH. Consequently, triggered by low iron, an IRP1-dependent feedback mechanism prevented HIF-2α–driven erythropoiesis, thus worsening iron deficiency as a whole (93). Notably, disruption of the IRP1–HIF-2α axis and IRP1-dependent feedback promoted PH development (94). Thus, Tempol-induced IRP1 activation has been proposed as an iron-specific therapy for PH (95). Clinical trials for iron replacement in PH are also ongoing (96–98). Yet, conversely, data in sickle cell disease have implicated iron overload as a causative trigger in PH (99). Disease context specificity and/or a need to maintain precise iron homeostasis may underlie these discrepancies, which in turn support caution in excessive iron repletion in PH patients.

Complementing altered iron homeostasis, deficiency of Fe-S clusters, bioinorganic cofactors required for enzyme redox potential (100), has been found to promote electron transport dysfunction and PH in experimental rodent models. Specifically, the hypoxia-induced microRNA miR-210 repressed the Fe-S cluster assembly proteins ISCU1 and ISCU2 (101, 102), attenuating Fe-S cluster integrity in endothelial cells. Coupled with these mechanistic data, exercise-induced PH (102) was identified in a patient with rare homozygous mutations of ISCU1/2. Deficiency of other Fe-S cluster assembly proteins may predispose to PH — an idea reinforced by patients with NFU1 (103) and BOLA3 mutations (104) who exhibit PH. Furthermore, IRP1 is Fe-S cluster–dependent, thus connecting the molecular controls of iron homeostasis in general with Fe-S cluster–driven metabolism and PH.

An imbalance in mitochondrial biogenesis and mitophagy

Mitochondrial number, or mass, governs cellular bioenergetic capacity and is regulated by biogenesis and mitophagy. Mitochondrial biogenesis is controlled by PPARγ coactivator (PGC1α), which activates nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) to promote mtDNA genes (105). PH models have demonstrated reduction in mitochondrial biogenesis genes and mass (22, 106, 107), but mechanistic understanding of biogenesis regulation is lacking. Multiple mediators affect mitochondrial biogenesis in PH, including NO/cGMP signaling (108–110). For example, PPHN modeled in fetal lambs resulted in decreased PGC1α, electron transport chain complex expression, and mtDNA copy number, which was reversed by NO donation (111). Therefore, mitochondrial biogenesis may be restored by treatment with NO derivatives, which are currently being investigated as therapies in PH (112). Separately, biogenesis markers may provide spatiotemporal information about PH pathogenesis. Monocrotaline-treated rats exhibited reduced expression of PGC1α, NRF1, and TFAM mRNA first in skeletal muscle and later in RV. Moreover, a concomitant decrease in mRNA and protein signatures appeared to differentiate decompensated versus compensated RV failure (113). Temporal perturbations in biogenesis such as these may underlie early-stage skeletal muscle–driven symptoms like exercise intolerance and late-stage heart failure symptoms. Ultimately, detailed temporal- and tissue-specific findings of mitochondrial biogenesis could facilitate stage-specific diagnostic and therapeutic intervention.

Imbalances in mitophagy, the process of removing damaged mitochondria, may also affect mitochondrial mass, but the role of mitophagy and autophagy in PH is incompletely understood (114). Uncoupling protein 2 (UCP2) is a calcium uniporter that maintains ER-dependent Ca^{2+} influx to the mitochondria, thus regulating activity of calcium-dependent proteins like PDH, ROS production, and mitochondrial-mediated apoptosis (28, 115, 116).

UCP2-deficient endothelial cells exhibited increased mitophagy and reduced mitochondrial mass (117). More work is required to understand how UCP2 and other factors may interconnect processes of biogenesis and mitophagy in PH.

Increased fission and mitochondrial fragmentation

Mitochondria exist in dynamic networks that are altered by fusion (joining) or fission (division). The dynamin-like GTPases mitofusin 1 and 2 (MFN1/2) mediate fusion, while translocation of activated dynamin-related protein 1 (DRP1) to the outer membrane mediates fission (118). Recent studies demonstrated enhanced mitochondrial fragmentation in PH. Animal models of PH showed increased DRP1 and mitochondrial fragmentation in PASMCs and RV that were reversed by treatment with the DRP1-targeting small molecule mitochondrial division inhibitor (Mdivi-1) or the FAO inhibitor trimetazidine (119–121). DRP1 translocation requires interaction with adaptor proteins (121), including the mitochondrial dynamics proteins MiD49 and MiD51. Elevated MiD49 and MiD51 levels in PASMCs from PAH patients led to enhanced DRP1-mediated fission (122). In parallel, decreased MFN2 expression was observed in diseased PASMCs (123) and skeletal muscle (6). Interestingly, MFN2 reciprocally regulated PGC1α, suggesting a link between diminished mitochondrial mass and increased fragmentation in PH (123). Mechanisms by which fission and fusion affect other cell types in PH are unknown, particularly in vivo.

In sum, disparate etiologies of PH seem to converge at the mitochondria, where certain pathologic features persist, including membrane hyperpolarization, altered ROS and iron handling, increased fragmentation, and decreased mitochondrial mass. Chronic mitochondrial perturbations influence metabolic reprogramming; however, the mechanism of mitochondrial and metabolic dysfunction remains unclear. Beyond genetic mutation and hypoxia, exploration of pathologic drivers in other PH subtypes may expand our understanding of mitochondrial and subsequent metabolic dysfunction in this disease.

Tissue-specific mitochondrial dysfunction

Pathologic changes associated with PH are found in several pulmonary vascular and extrapulmonary cell types. Here, we address nuanced differences in metabolic rewiring in the RV–pulmonary artery (RV-PA) circuit. Additionally, while prior studies focused on tissues with high bioenergetic requirement and mitochondrial content, like PASMCs, we explore the increasingly more relevant roles of mitochondria in other cell types.

The right ventricle. Similar to other cardiopulmonary cell types, RV cardiomyocytes exhibit metabolic rewiring (i.e., increased glycolysis, glutaminolysis, and FAO at the expense of glucose oxidation) in PH, as discussed above (39, 124). RV dysfunction may be driven by stepwise, rather than continuous, shifts from normal myocardium to adaptive and maladaptive remodeling. Initial studies have shown distinct metabolic phenotypes between normal, hypertrophic, and decompensated RV failure — namely, increased glycolysis and reduced glucose oxidation as a response to hypoxia during cardiomyocyte hypertrophy and more complex alterations in glucose and FA oxidation in decompensated RV failure (19, 124–126). Additionally, while HIF-1α expression between the compensated and decompensated RV did not change, HIF-1–dependent genes were differentially expressed (19). In sum, the RV appears to exhibit a distinct stepwise metabolic program, often independent from chronic pressure overload due to pulmonary vascular remodeling (127). Temporal assessment of RV function by novel, noninvasive imaging modalities like PET may be required to better distinguish this metabolic shift and to ensure timing and efficacy of metabolic treatments.

Endothelial cells. Endothelial dysfunction in PH is complex and not fully understood; a prominent model asserts that initial injury and apoptosis precede pathogenic hyperproliferation in PH (128, 129). Understanding the dynamic phenotype(s) of the pulmonary endothelium has significant clinical implications, potentially facilitating diagnostic determination of disease stage and therapy. Given that endothelial dysfunction in peripheral and pulmonary circuits is defined by rewiring of multiple metabolic pathways (16, 40, 58, 130, 131), it is conceivable that mitochondrial changes may provide context for these spatiotemporal alterations. Study of endothelial cells that are genetically predisposed to PH has provided some clues. Transgenic and heterozygous *Bmpr2*-mutant mice exhibited increased mtDNA damage in the form of peroxidation-induced DNA adducts (81). In patients with BMPR2 haploinsufficiency, heritable PAH is linked to endothelial metabolic dysfunction (16, 130). BMPR2-mutant endothelial cells displayed increased p53, mitochondrial biogenesis gene expression, and glycolytic flux, resulting in a proinflammatory state that predisposes to PH. Conversely, BMPR2-deficient endothelial cells exposed to hypoxia followed by reoxygenation demonstrated diminished p53-driven mitochondrial biogenesis coupled with increased mitochondrial fission, mtDNA damage, and apoptosis. Both pathways illustrate how BMPR2 mutation and resultant perturbations in mitochondrial dynamics increase susceptibility to endothelial dysfunction and PH (132). Further interrogation of endothelial mitochondrial function could be instrumental in characterizing the maladaptive shift between early-stage apoptosis and potentially late-stage phenotypes like endothelial-mesenchymal transition (72, 133) or hyperproliferation (2, 128, 134). The study of endothelial cells derived from BMPR2-haploinsufficient inducible pluripotent stem cells may facilitate valuable insights (135–137).

Tissues anatomically distinct from the RV-PA circuit. Cells in circulation — erythrocytes, platelets, and immune cells — are also a focus of study in PH, but how mitochondrial defects in hematopoietic cells influence the pulmonary vasculature is not fully defined. PH is often a complication of hematologic disorders, particularly anemias (138). In that context, pathologic and age-related drivers of mtDNA mutagenesis are known to prevent mitochondrial elimination from reticulocytes, resulting in deregulated erythroid maturation (139, 140). It is unknown whether erythroid progenitor mitochondrial dysfunction could predispose to PH. Similarly, platelets from PAH patients exhibit increased glycolytic flux and OXPHOS (61), but a causative link between platelet metabolic dysfunction and PH is not established. Finally, perivascular inflammation is a common feature of PH (7), and immune cell recruitment to the pulmonary vasculature is under substantial study (141–143). While metabolic reprogramming is linked to macrophage plasticity (144) and T cell activation (145), a direct connection between that metabolic rewiring and pulmonary vascular inflammation has yet to be defined (146). Furthermore, damage-induced mtDNA release was shown to augment a damage-associated molecular pattern–medi-

ated (DAMP-mediated) innate immune response in other diseases (147), and DAMP-dependent responses in PH are currently under investigation (81).

The role of progenitor cells in PH is also an evolving field of study (148, 149), and mitochondrial dysfunction in these cells may play a prominent role. Prior data suggested that progenitor cells in PH exhibited altered glucose metabolism; namely, blood outgrowth endothelial cells displayed increased glycolysis (150), and $CD133^+$ progenitor cells showed increased G6PD expression and flux through the PPP (33). Shifting endothelial progenitor cells toward mitochondrial OXPHOS was shown to be protective after ischemic injury (151); similar processes may protect against PH.

Given the breadth of metabolic reprogramming across multiple cell types in PH, studies of mitochondrial dysfunction may eventually implicate other anatomically distinct tissues in PH pathogenesis. In particular, there may be mitochondrial-specific changes in the brain, gut microbiome, and liver, especially in portopulmonary hypertension and iron deficiency (152). Additionally, with the advent of high-throughput methods like single-cell RNA sequencing, nuanced differences in metabolism and mitochondrial function among cells in the same tissue can now be discerned. Lastly, given the multitissue mitochondrial dysfunction in PH, an attractive model may be emerging whereby novel endocrine processes could control anatomic coordination of mitochondrial dysfunction, potentially spearheaded by secretion and uptake of molecular messengers like mitokines (153) or microRNAs, already known to be important in this disease.

Evolving translational technologies

Diagnostics for metabolic reprogramming

Imaging. While hemodynamic monitoring remains the gold standard, diagnostic modalities that provide early, accurate, and noninvasive measurement of tissue-specific pathology are needed. The Warburg effect provides the basis for adopting ^{18}F-fluorodeoxyglucose PET (FDG-PET) as a diagnostic tool for PH (154–156). FDG-PET is already used in the clinical setting in other diseases and may provide quantitative spatiotemporal measurement of metabolic reprogramming in RV to complement current hemodynamic testing. Moreover, it may be a future tool for longitudinal measurement of treatment response and targeted drug delivery to metabolically dysregulated tissues. Use of FDG-PET may also prompt adaptation of PET for other metabolically relevant markers in PH-related pathology, such as FAs, amino acids, and TCA metabolites like L-2-hydroxyglutarate, which coordinate pulmonary vascular metabolic responses to reductive stress (157). Metabolic disease progression in the RV may also be followed by four-dimensional flow cardiac MRI, which illustrates RV energy work density and energy dissipation (158), and hyperpolarized cardiac MRI, which can quantify muscle-specific metabolites (159). PET and cardiac MRI can even be coupled in a hybrid technology (160) that may provide an unprecedented view of metabolic perturbations in compensated versus decompensated RV failure, allowing for better diagnostic and prognostic detail in PH patients.

Plasma metabolomics. Thus far, circulating metabolite screens in PH have aimed to identify unique profiles in PH patients and/or distinct PH subtypes for diagnostic and prognostic benefit. Data suggest that certain microRNAs (102, 161), plasma acylcarnitines (47, 162), glutamate (163), TCA intermediates, amino acids (163, 164), transfer RNA–specific modified nucleosides (163), purines, indoleamines, and indoleamine 2,3-dioxygenase–dependent tryptophan metabolites (165) are differentially expressed in plasma among various cohorts of PH patients. Moreover, significant metabolite alterations prognosticated risk of death (163) and correlated with hemodynamic measurements (165). High-throughput screening of patient plasma in tandem with other diagnostic tools, like that proposed in the US National Heart, Lung, and Blood Institute's Pulmonary Vascular Disease Phenomics Program (PVDOMICS) initiative (166) and the UK National Cohort Study of PAH (163), could provide group- and stage-specific information and be a step toward implementing precision metabolomic medicine in PH. However, elucidating site-specific metabolic dysregulation via metabolomics may require more invasive transpulmonary blood sampling to ascertain cell source (165). As is the case in recent analyses (163, 166), studies also require large-scale cohort validation of existing data. Additional barriers to implementation of routine metabolomic screening include the lack of standardized methods for sampling and detection, controlling among experiments, and interpretation.

Together, next-generation metabolic imaging and metabolomic biomarkers could improve early diagnosis and longitudinal management of PH. Yet a better understanding of the underlying pathology, large-scale analyses correlated with clinical metrics, and removal of high cost and barriers to access are all required for successful implementation.

Prospective mitochondria-targeted therapies

Mitochondria-specific therapies have the potential to slow or regress PH development, but several challenges remain. First, tested therapies result in heterogeneous responses in PH patients. Dichloroacetate (DCA) inhibits PDK2 and upregulates PDH activity, restoring glucose oxidation in PASMCs, endothelial cells, and RV (50, 124). Ultimately, this therapy prevented and reversed PH development in animal models and certain PAH patients (25, 167–169). Notably, a subset of patients exhibited variable responses to DCA treatment — heterogeneity that appeared to be dictated by loss-of-function genetic polymorphisms in sirtuin 3 (SIRT3) and UCP2 (168). These data indicate that precision medicine may improve selection of appropriate therapy for patients. Importantly, single-drug metabolic therapy may not be sufficient to reverse PH pathology given dysregulation of numerous metabolic pathways across multiple affected tissues. For example, ranolazine, the FAO inhibitor, improved PAH symptoms and RV function (57) despite the lack of hemodynamic improvement in MPAP (57). It is possible that ranolazine's efficacy may be limited to the RV, with unclear precedent for interpreting such data and their clinical implications. To address these obstacles more efficiently, we contend that immediate investment is imperative for obtaining *-omics*-level genetic, transcriptomic, and metabolomic data from clinical trials as combination therapies are tested. Such comprehensive data will be essential in deconvoluting the complexity of these metabolic shifts in PH patients and will eventually guide creation of more effective mitochondrial-specific therapies, as depicted in Figure 2.

Redefining metabolic overlap in subtypes of PH

Metabolic syndrome and PH

The clinical classification system for PH defines subtype based on presumed etiology, with consequences for prognosis and therapy. However, given the cumulative data on metabolic and mitochondrial etiologies in PH, we have considered whether this schema must evolve to reflect the intersection or divergence of metabolic phenotypes among PH subtypes. An example of this concept is the association of metabolic syndrome, heart failure with preserved ejection fraction (HFpEF), and PH. Cardiovascular disease has long been linked to metabolic syndrome — dyslipidemia, systemic hypertension, elevated fasting glucose levels, and central obesity (170). Only recently was PH connected to metabolic syndrome (171, 172) with patients exhibiting dyslipidemia (173) and insulin resistance (174, 175). In addition, SIRT3 (176) and UCP2 (177) polymorphisms are associated with metabolic syndrome and PH (168, 178). Tissue-specific SIRT3 knockdown and subsequent metabolic reprogramming were studied in PASMCs (178), endothelial cells (130), fibroblasts (18), and skeletal muscle (179). Interestingly, early restoration of SIRT3 expression in skeletal muscle, the predominant site of insulin-mediated glucose uptake (180), by nitrite or metformin reversed insulin resistance and reduced pulmonary pressures and vascular remodeling in a rodent model of metabolic syndrome and HFpEF (179). This study confirms the importance of skeletal muscle in metabolically driven PH and illustrates the role of metabolic syndrome in PH. Yet classification of group 2 PH or PH-HFpEF does not reflect this distinct molecular characteristic. Specifically, data support that metabolic syndrome may be present in all PH subtypes, with a majority of patients in groups 2 and 3 PH (172). Furthermore, HFpEF itself is a diverse clinical entity that encompasses patients with LV diastolic dysfunction without metabolic syndrome. Therefore, we expect that molecular classification of PH subtypes will be an evolving concept, particularly as we learn more about deregulated metabolism in this disease.

Aging and PH

The age of patients diagnosed with PH has steadily risen in recent decades (181), and interest in age-associated alterations in pulmonary vascular function has grown (182, 183). Moreover, several similarities have emerged between mitochondrial dysfunction during aging and PH progression. Investigating these perturbations through the lens of aging may deepen and broaden our understanding of the role of mitochondria in PH. For example, stem cell niche and cellular senescence are largely driven by age-dependent alterations in mitochondrial metabolism (184), yet the effect of these processes on the pulmonary vasculature is largely undefined. Without a pathologic aging model of PH and with a limited number of longitudinal PH databases, the extended experimental timeline has prevented study of aging and PH. These challenges are not insurmountable, and we expect that future exploration of the impact of aging on PH will also influence its clinical classification and provide valuable opportunities to adapt PH management in a more elderly population.

Conclusion

Intensified investigation has revealed the complex metabolic reprogramming and perturbations in mitochondrial dynamics in PH within and beyond the RV-PA circuit. Yet fundamental questions still remain, including the causal mechanism of mitochondrial dysfunction, how these dysregulated metabolic pathways work synergistically or in opposition to promote disease, and how tissue-specificity and disease stage overlay and define these metabolic programs. Diagnostic and therapeutic technologies based on common mitochondrial features in PH are promising but require further development in tandem with high-throughput *-omics* evaluation, given disease heterogeneity. With mitochondrial dysfunction as a central driver of PH, we expect new, causative, and surprising connections to emerge between PH and other mitochondria-dependent diseases. These links will likely provide a basis for refined classification of PH subtypes in order to move closer to a precision medicine approach for diagnosis and management of this historically neglected disease.

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