Recent Advances in Pulmonary Hypertension

Emerging Concepts in the Molecular Basis of Pulmonary Arterial Hypertension

Part I: Metabolic Plasticity and Mitochondrial Dynamics in the Pulmonary Circulation and Right Ventricle in Pulmonary Arterial Hypertension

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Mitochondria are central to cellular metabolism. The metabolic pathways of mitochondria include fatty acid oxidation, glucose oxidation, and glutaminolysis. The initial step in glucose metabolism occurs in the cytosol, where glycolysis converts glucose to pyruvate (Figure 1).

Normally, glycolysis is coupled to glucose oxidation, meaning that the pyruvate is transported into the mitochondria, where it serves as a substrate for pyruvate dehydrogenase (PDH). Under pathological conditions such as inhibition of PDH, glycolysis may be uncoupled from glucose oxidation and remain a wholly cytosolic reaction that terminates in the generation of lactate.

Metabolism is quite plastic, and the relative importance of each pathway can change in response to environmental stimuli such as substrate availability and the developmental stage of the organism and pathological stimuli such as hypoxia, shear stress, pressure overload, ischemia, and hypertrophy. In addition, the activity of one metabolic pathway alters the activity of competing pathways. Examples of this metabolic cross-talk include the reciprocal relationship between fatty acid and glucose oxidation. Fatty acid oxidation suppresses glucose oxidation through a mechanism called the Randle cycle (Figure 2), named after Phillip Randle, who first described the phenomenon. Another example of metabolic plasticity is the uncoupling of glycolysis from glucose oxidation, so-called aerobic glycolysis. Aerobic glycolysis is also called the Warburg effect, in honor of Otto Warburg, who first described the phenomenon in cancer cells. Warburg et al noted that this shift to glycolysis contributed to the growth and survival advantage of cancer cells. They also observed, but could not explain, accumulation of ammonia in their cancer tissue cultures.

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In addition to generating ATP, mitochondria are constantly dividing and joining together. These highly conserved and regulated processes are called fission and fusion, respectively. These noncanonical mitochondrial functions (fission and fusion), as well as migration, are called mitochondrial dynamics. Mitochondrial dynamics are important in physiology, participating in oxygen sensing and distributing mitochondria to daughter cells during mitosis. Mitochondrial dynamics are also involved in cellular quality control, notably participating in mitophagy and apoptosis. Acquired and inherited disorders of mitochondrial dynamics are involved in diseases, including pulmonary arterial hypertension (PAH), cancer, and cardiac ischemia/reperfusion injury. Both metabolic plasticity and mitochondrial dynamics are relevant to the pathogenesis of PAH and offer new therapeutic targets in the pulmonary vasculature and the right ventricle (RV).

Mitochondria and Metabolism in PAH

Vascular cells and RV cardiomyocytes in PAH have a mitochondrial-metabolic phenotype similar to that seen in cancer. The cancer-like metabolic phenotype in PAH includes increased energetic reliance on aerobic glycolysis; inhibition of mitochondrial respiration resulting from pathological activation of transcription factors such as cMyc, Forkhead transcription factor (Forkhead box protein O1), and hypoxia-inducible factor (HIF-1α); and PDK-induced PDH inhibition. In the hypertrophied RV, cancer-like metabolic changes, aerobic glycolysis and glutaminolysis, reduce energy production and contractility. PAH vascular cells and cancer cells also share a mitochondrial morphological phenotype (increased mitochondrial fragmentation) that is caused by a fission/fusion imbalance. Mitochondrial fragmentation contributes to the proliferative, apoptosis-resistant phenotype of both diseases.

Although the analogy between PAH and cancer is imperfect, both syndromes share a propensity for cell enlargement, proliferation, and apoptosis resistance that is attributable in...
part to acquired disorders of mitochondrial metabolism and mitochondrial dynamics. Preclinical studies in rodent models of PAH have identified the therapeutic benefits of targeting these mitochondrial abnormalities in the lung to regress vascular obstruction and improve hemodynamics and in the RV to improve contractility, increase cardiac output, and reduce hypertrophy. In this review, we summarize the mechanism of several mitochondrial abnormalities in PAH and discuss potential therapeutic targets in the pulmonary vasculature and RV. Readers are referred to several recent reviews on the subject of metabolism and mitochondrial dynamics in PAH.

### A Brief Review of Metabolism

In the fetus, glucose oxidation and glycolysis are the major sources of cardiac ATP, and circulating levels of free fatty acids are low. In the adult heart, the predominant energy source is fatty acid oxidation (60%–90%); however, glucose metabolism continues to contribute to ATP production. Although it is classically considered a secondary source of ATP production in the adult heart, direct measurement shows that glucose oxidation remains an important source of ATP in the normal RV, accounting for 48% of total ATP produced.

Despite the complexities of the metabolic pathways, glucose oxidation, fatty acid oxidation, and glutaminolysis have some common features. First, each pathway imports its substrate through a transporter into the cytosol. The transporters for glucose, fatty acids, and glutamine are the glucose transporters (Glut 1–4), the fatty acid transport proteins 1 and 6, and the solute carrier proteins (SLC 1A5 and 7A5), respectively (Figures 1–3). When substrate use is increased, transporter expression rises, as is the case for Glut and SLC1A5 in the hypertrophied RV in PAH.

**Figure 1.** Mechanism of impaired glucose oxidation and enhanced aerobic glycolysis in pulmonary arterial hypertension. Changes in redox signaling such as downregulation of superoxide dismutase 2 (SOD2) and the resultant decrease in hydrogen peroxide (H₂O₂) signaling can activate transcription factors (ie, hypoxia-inducible factor-1α [HIF-1α]), which in turn upregulate pyruvate dehydrogenase (PDH) kinase (PDK). PDK inhibits PDH, which impairs oxidative glucose metabolism, causing the cell to rely on other forms of metabolism, such as aerobic glycolysis. Dichloroacetate, the small-molecule inhibitor of PDK, can reactivate PDH and restore oxidative glucose metabolism. ETC indicates electron transport chain; FOXO1, Forkhead box protein O1; HK, hexokinase; LDHA, lactate dehydrogenase A; PDK, phosphofructokinase. Reprinted from Piao et al with permission from the publisher. Copyright © 2010 Springer International Publishing AG.

**Figure 2.** Manipulating fatty acid and glucose oxidation in pulmonary arterial hypertension: the Randle cycle. The Randle cycle is the reciprocal relationship between glucose oxidation and fatty acid oxidation. Note how the acetyl CoA and citrate produced by β-oxidation of fatty acids inhibit pyruvate dehydrogenase (PDH) in the mitochondria and phosphofructokinase (in the cytosol). This feedback (along with other indicated feedback mechanisms) slows glucose oxidation under conditions in which there is substantial fatty acid oxidation. Trimetazidine and ranolazine, the pharmacological inhibitors of fatty acid oxidation, can restore glucose oxidation by partially inhibiting fatty acid oxidation and activating the Randle cycle. CPT1/2 indicates carnitine palmitoyltransferase 1/2; FA-CoA, fatty acyl-CoA; FATP1/6, fatty acid transport protein 1/6; Glut1/4, glucose transporter 1/4; HK, hexokinase; IMM, inner mitochondrial membrane; LDHA, lactate dehydrogenase A; OMM, outer mitochondrial membrane; RAN, ranolazine; and TMZ, trimetazidine. Reprinted from Fang et al with permission from the publisher. Copyright © 2012 Springer International Publishing AG.
pathways drives the Krebs cycle and promotes ATP production. The glucose and fatty acid oxidation pathways ultimately increase Acetyl CoA levels and provide the electron donors that fuel the majority of cellular ATP generation. Third, it appears that most of the pathways display cross-talk such that when one is increased another is depressed. This reciprocal relationship is well established for glucose and fatty acid oxidation (the Randle cycle); however, it also appears to be the case for glutaminolysis and glucose oxidation, although independent corroboration is required. Fourth, it appears that most of the metabolic changes seen in the RV and pulmonary artery in PAH are maladaptive in that metabolic modulators that restore depressed glucose oxidation or inhibit upregulated fatty acid oxidation and glutaminolysis are beneficial to the hemodynamic and functional states of the animal.

Despite these commonalities, the pathways vary greatly in their bioenergetic yield. A fatty acid containing 6 carbons subjected to β-oxidation in the mitochondria can yield 48 ATPs. However, fatty acid oxidation comes at a price in that it uses ≈10% more oxygen than glucose oxidation to generate the same amount of ATP (Figure 2). The energetic premium associated with fatty acid oxidation reflects the inhibition of glucose oxidation via the Randle cycle, which leads to aerobic glycolysis and lactate accumulation. The resulting acidosis must be corrected by transporters and pumps at an energetic cost. In contrast, although oxidation of 6 carbon–containing glucose has lower ATP yield, it is more efficient in that it does not elicit aerobic glycolysis and does not engender an excess production of lactate. In aerobic glycolysis, only 2 ATPs are generated per mole of glucose, and lactate is produced.

Metabolism of the amino acid glutamine via glutaminolysis also occurs in PAH and cancer. In glutaminolysis, glutamine is hydrolyzed to glutamate and converted to α-ketoglutarate in the mitochondria. α-Ketoglutarate then enters the Krebs cycle and replenishes metabolic intermediates, thereby supporting rapid cell growth. In addition, glutaminolysis can increase nitrogen anabolism, which further supports cell growth. Glutaminolysis was originally identified in cancer cells but has recently been found to be induced in the heart during RV hypertrophy (RVH; Figure 3). In the RV, glutaminolysis generates modest energy but rewards cells with amino acid intermediates that permit rapid cell growth.

A Brief Review of Mitochondrial Dynamics

Mitochondrial fusion is mediated by large GTPases, mitofusin-1 and mitofusin-2, which reside in the outer mitochondrial membrane, and a GTPase called optic atrophy-1 in the inner mitochondria membrane. Fission is mediated by the GTPase dynamin-related protein 1, which on activation moves from the cytosol to the outer mitochondrial membrane.

**Figure 3.** Proposed mechanism of glutaminolysis in the hypertrophied right ventricle (RV). RV ischemia and capillary rarefaction activate cMyc and its binding partner, Max, which increases the expression of the glutamine transporters (SLC 1A5 and 1A7) and augments glutamine uptake. This drives the production of α-ketoglutarate (α-KG). α-KG enters the Krebs cycle, leading to production of malate. The Krebs cycle–derived malate generates cytosolic pyruvate, which is converted by lactate dehydrogenase A (LDHA) to lactate. In conditions of high glutaminolysis, glucose oxidation is inhibited. 6-Diazo-5-oxo- L-norleucine (DON) can inhibit glutaminolysis and restore glucose oxidation. Hypoxia-inducible factor-1α (HIF-1α) increases the transcription of the some of the same glycolytic mediators as cMyc and Max, notably glucose transporter 1 (Glut1) and hexokinase (HK) 2. GLS indicates glutaminase; LDHA, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; and PFK, phosphofructokinase. Reprinted from Piao et al21 with permission from the publisher. Copyright © 2012 Springer International Publishing AG.
There it interacts with non–GTPase-binding partners such as mitochondrial fragmentation factor and fission factor 1, resulting in multimerization and division of the mitochondria (reviewed elsewhere’).

Many of the abnormalities that occur in PAH promote fission, notably increased intracellular calcium, increased activity of the mitosis promoter cyclin B1/CDK1, and normoxic activation of HIF-1α.10 Inhibition of mitochondrial fission, achieved by administration of inhibitors of dynamin-related protein 1 such as mdivi-1, regresses PAH in rodent models by arresting pulmonary artery smooth muscle cells in the G2/M phase of the cell cycle and promoting apoptosis.10 Mitochondrial fusion is also decreased in PAH. The decrease in mitochondrial fusion reflects in part a reduced expression of both mitofusin-2 and its transcriptional coactivator, peroxisome proliferator-activated receptor-γ coactivator 1-α.12 Augmentation of mitofusin-2 expression is antiproliferative and proapoptotic and improves hemodynamics in rodent PAH models.12 Although the linkage between mitochondrial dynamics and metabolism is poorly understood in PAH, there are examples in which form and function clearly intersect. For example, in skeletal muscle cells, decreases in mitofusin-2 reduce glucose oxidation and oxygen consumption.24 In myotubes, decreases in mitofusin-2 similarly reduce the oxidation of pyruvate, palmitate, and glucose.25 Thus, mitofusin-2 deficiency may contribute to the glycolytic shift observed in pulmonary artery smooth muscle cells in PAH (Figure 4).10,12,26

**Impaired Oxygen Sensing and Normoxic HIF-1α Activation in the Pulmonary Vasculature in PAH**

The mitochondria in pulmonary artery smooth muscle cells normally serve as oxygen sensors.10 However, in PAH, there is normoxic activation of HIF-1α, which creates a “pseudo-hypoxic” environment despite normal oxygen availability.26 The term pseudohypoxia conveys the concept that changes in mitochondrial metabolism and redox signaling normally seen in response to environmental hypoxia are occurring despite adequate oxygen supply. In the lung, the pseudohypoxic state is associated with impairment of a well-established mitochondria–reactive oxygen species (ROS)–HIF-1α–Kv1.5 oxygen-sensing pathway.26 In PAH, downregulation of the mitochondrial hydrogen peroxide (H2O2)–generating enzyme superoxide dismutase 2 (SOD2) decreases production of the redox signaling molecule H2O2, thus creating an hypoxia-like redox milieu that activates HIF-1α.27 HIF-1α in turn transcriptionally upregulates PDK, which inhibits PDH and further reduces the production of ROS by the mitochondrial electron transport chain. Loss of physiological levels of ROS inhibits and downregulates the expression of the oxygen- and voltage-sensitive potassium channel Kv1.5, resulting in depolarization and calcium overloading of the smooth muscle cells. Thus, the mitochondria–ROS–HIF-1α–Kv1.5 oxygen-sensing pathway is subverted in PAH, contributing to downstream changes in mitochondrial metabolism and dynamics.

Pseudohypoxia can be modeled in cell culture by activating hypoxic transcriptional pathways in a P0-, independent manner. For example, adenoviral overexpression of constitutively activated HIF-1α in normoxic human arterial endothelial cells leads to overexpression of hundreds of hypoxia-responsive genes.28 This concept of pseudohypoxia in PAH suggests the feasibility of treatments directed at either restoring oxygen sensing (targeting the mitochondrial electron transport chain and SOD2) or correcting the downstream mitochondrial-metabolic abnormalities that result from impaired oxygen sensing.14

The interrelatedness of disorders in mitochondrial oxygen sensing and metabolism is shown in Figure 1. One example of an acquired but heritable mechanism by which the mitochondrial metabolic changes of PAH can occur is the epigenetic inhibition of the expression and activity of SOD2.27,28 SOD2 is a nuclear-encoded, mitochondrial enzyme responsible for the production of the diffusible, redox-signaling molecule H2O2.26,27,30 Loss of SOD2-mediated production of H2O2 activates HIF-1α.10,27 The relationship between SOD2 and HIF-1α is robust. Simply downregulating SOD2 in normal pulmonary artery smooth muscle cells with small inhibitory RNA activates HIF-1α despite normal P0. Likewise, once HIF-1α is activated, there is a clear phenotypic shift in pulmonary artery smooth muscle cells, which become hyperproliferative and display increased mitochondrial fragmentation.10,12 The importance of epigenetic inhibition of SOD2 and activation of HIF-1α was first identified in the Fawn hooded rat, a strain that spontaneously develops PAH.26 HIF-1α activation in smooth muscle cells from Fawn hooded rats and PAH patients persists in culture despite abundant O2.27

Transcriptional repression of SOD2 expression occurs through methylation of 2 key CpG islands in the SOD2 gene.
Methylation in the promoter and enhancer regions of the gene halves SOD2 expression, and the resulting reduction of H$_2$O$_2$ production initiates HIF-1α activation. Prolonged activation of HIF-1α and the associated mitochondrial fragmentation promotes a glycolytic shift in metabolism and hyperproliferation of pulmonary artery smooth muscle cells. In Fawn hooded rats, demethylation of the SOD2 gene by the DNA methyltransferase inhibitor 5-azacytidine restores SOD2 expression and inhibits pulmonary artery smooth muscle cell proliferation. It remains unclear why the dysregulation of DNA methyltransferases in the Fawn hooded rat is confined to the lung, although there is selective upregulation of DNA methyltransferases in the lung that is not seen in the systemic vasculature. Interestingly, epigenetic inhibition of SOD2 also contributes to the hyperproliferative phenotype of some cancer cells.32,33

Additional evidence implicating HIF-1α in the pathogenesis of PAH comes from Chuvash pulmonary hypertension. This syndrome affects individuals in the mid Volga river region of Russia. They develop a hypoxic phenotype that is characterized by polycythemia and pulmonary hypertension resulting from inappropriate normoxic activation of HIF-1α and increased expression of genes for erythropoietin, Glut1, and vascular endothelial growth factor (VEGF).34 They also have PDK activation and elevated plasma lactate levels. This pseudohypoxic pathophysiology is similar to that of the Fawn hooded rat but results from a homozygous mutation in the von Hippel-Lindau gene (VHL 598C-T) that removes the ubiquitination signal for HIF-1α degradation35 and thus impairs proteasomal degradation of HIF-1α. Further implicating a role for HIF-1α activation in PAH is the observation that normoxic cobalt-induced HIF-1α activation causes mitochondrial fragmentation within 2 to 3 hours. Interestingly, cobalt does not cause cell proliferation in culture, likely as a result of nonspecific toxicity. However, long-term in vivo administration of cobalt engenders pulmonary hypertension and RVH with evidence of HIF-1α activation and increased mitochondrial fission in the pulmonary artery smooth muscle cells.10 Furthermore, inhibitors of HIF-1α, for example, chetomin, can reverse the hyperproliferative metabolic effects of this transcription factor in PAH.31 Finally, the role of HIF-1α in PAH is also evident from the observation that HIF-1α haploinsufficiency disrupts oxygen sensing and reduces hypoxic pulmonary hypertension in mice.6,37

**Metabolic Remodeling in the Hypertrophied RV in PAH**

Altered RV metabolism in PAH is transcriptionally mediated, although posttranscriptional roles for microRNAs are increasingly being recognized. However, in the RV, the likely precipitant is ischemia rather than impaired oxygen sensing. In RVH, there is ischemia with decreased coronary flow reserve; however, it is unclear whether this reflects impaired epicardial perfusion pressure, capillary rarefaction, or both. The right coronary artery normally fills during both systole and diastole because the RV systolic pressure is low relative to the driving pressure in the aorta. In RV pressure overload, the systolic perfusion gradient between the aortic and RV systolic pressures may disappear, limiting right coronary artery flow to diastole. This essentially halves the amount of blood being supplied to the hypertrophied RV, which has increased metabolic demands.40 At extremes of pulmonary hypertension, when right coronary artery perfusion pressure falls below 50 mm Hg, RV contractile function declines.41 RV ischemia in PAH may also result from impairment in angiogenesis, also referred to as capillary rarefaction (Figure 5A). The impairment in angiogenesis may result from decreased expression of genes such as insulin-like growth factor 1, VEGF, apelin, and angiopeptin-1 (Figure 5B).21 Occlusive microvascular disease and capillary rarefaction have been seen in the RV in animal models of maladaptive PAH30,42 and in patients with scleroderma-associated PAH (Figure 5C).21

The role played by HIF-1α in the metabolic remodeling of the RV in PAH is less clear than its role in the pulmonary vasculature. Several investigators have found that HIF-1α is increased in rodent RVH models.13,44 However, there are differences in the role of HIF-1α in the ventricle versus the lung in terms of the predominant downstream PDK isoform expression profile that is elicited and in the temporal profile of HIF-1α (reviewed in the Controversies section).

**A Central Role for Inhibition of PDH in PAH**

In PAH and cancer, PDH is phosphorylated and inhibited by PDK. PDK expression is increased in these syndromes. Phosphorylation of the α-subunit of the E1 (pyruvate decarboxylase) component of the PDH complex by any PDK isoform rapidly inhibits PDH. When PDH is inhibited by PDK, the supply of electron donors to the Krebs cycle is limited, and energy production is reduced45 (Figure 1).

The 4 PDK isoforms differ in their transcriptional regulation and tissue distribution.46 PDK2 appears to be the predominant human isoform in many tissues; however, the magnitude and consequences of regional isoform heterogeneity among tissues have not been adequately studied. For example, PDK1 expression is transcriptionally upregulated by HIF-1α20,47 whereas PDK4, which lacks a hypoxia recognition element in its promoter and thus is not directly regulated by HIF-1α, is induced by Forkhead box protein O1.20,48 However, HIF-1α can transcriptionally upregulate estrogen-related receptor γ, which is capable of transcriptionally increasing PDK4 expression.49

The dominant PDK isoforms in a specific tissue can change with disease, and this pathological isoform variation is largely unstudied. For example, in rodent PAH, the dominant PDK isoforms upregulated in the RV are PDK2 and PDK4.20 Anecdotal evidence suggests that PDK4 is also upregulated in the human RV.50 In the lung, the predominant isoforms upregulated in PAH are PDK1 and PDK2.1,2,51–53

Tissue heterogeneity in PDK expression and disease-specific regulation of PDK and PDH in PAH merit further study. In some tissues, there appears to be minimal basal PDK activity, whereas in others, PDK is active under physiological conditions. In skeletal muscle, tonic activation of PDK2 contributes to the regulation of carbohydrate oxidation and the production of reducing equivalents for the electron transport chain.47 In contrast, there appears to be little tonic PDK activity in the normal RV and pulmonary artery, as indicated by the absence of effect of a pan-PDK inhibitor, dichloroacetate
When the mitochondrial PDH complex is active, pyruvate is converted to acetyl coenzyme A, which fuels the Krebs cycle, generating electron donors for the electron transport chain and fueling generation of ATP. However, in PAH, PDH inhibition inhibits the electron transport chain and increases reliance on aerobic glycolysis. This metabolic shift contributes to the hyperproliferative, apoptosis-resistant state of pulmonary artery smooth muscle cells. The PDK-mediated metabolic switch to aerobic glycolysis is associated with decreased cardiac output and reduced RV contractility. Increased lactate production further impairs RV function secondary to acidosis. Suppression of glucose oxidation reflects the acceptance by the cell of reduced efficiency of ATP generation in exchange for reduced risk of mitochondria-mediated apoptosis and an increased ability to hypertrophy.

Increased glycolysis is associated with increased glucose flux, allowing RV glycolysis to be detected on cardiac 18F-fluorodeoxyglucose (18FDG) positron emission tomography (PET) scans in patients and in animal models. There is also preliminary evidence that a reduction in RV afterload by initiation of pulmonary vasodilators reduces RV uptake of 18FDG in patients. In a case report comparing a PAH patient who died of rapidly decompensating RV failure with a long-term survivor, expression of both PDK4 and the Glut1 transporter was more elevated in the patient with the rapidly fatal RVH. Dichloroacetate is a small-molecule pyruvate analog. Dichloroacetate inhibits all 4 PDK isoforms by binding a conserved, allosteric site in the N-terminal domain. The dichloroacetate-binding pocket is relatively small (volume, 211 Å³), and it is buried within the PDK structure, making it relatively inaccessible to molecules larger than pyruvate. These allosteric constraints make it difficult to modify dichloroacetate to enhance its potency. Consequently, recent studies have attempted to inhibit PDK by targeting its larger (volume, 865 Å³), more accessible ATP-binding pocket. Dichloroacetate-induced metabolic changes depolarize mitochondria and induce apoptosis while inhibiting pulmonary artery smooth muscle cell proliferation. Dichloroacetate is relatively specific for abnormal tissues and has little effect on normal cardiac or vascular cells, in which PDK isoforms are inactive. Oral dichloroacetate is effective in regressing pulmonary vascular disease and improving RV function in preclinical models of pulmonary hypertension, including chronic hypoxic pulmonary hypertension. Oral dichloroacetate is effective in regressing pulmonary vascular disease and improving RV function in preclinical models of pulmonary hypertension, including chronic hypoxic pulmonary hypertension.

Figure 5. Adaptive vs maladaptive forms of right ventricular (RV) hypertrophy (RVH). A, Catheterization data show simultaneous RV pressure and aortic pressure (AoP) in various rat RVH models. Despite similar coronary perfusion pressure (defined as the ΔAoP−RV), there are worse RV function and exercise capacity in monocrotaline (MCT) vs pulmonary artery banding (PAB; not shown). B, Representative images and mean data showing greater RV capillary rarefaction (loss of small arteries) in a maladaptive form of RVH (MCT) than in an adaptive form of RVH (created by PAB). Reprinted from Piao et al with permission from the publisher. (Figure 1), on metabolism and the cellular electrophysiology of normal RV and pulmonary vascular cells. When the mitochondrial PDH complex is active, pyruvate is converted to acetyl coenzyme A, which fuels the Krebs cycle, generating electron donors for the electron transport chain and fueling generation of ATP. However, in PAH, PDH inhibition inhibits the electron transport chain and increases reliance on aerobic glycolysis. This metabolic shift contributes to the hyperproliferative, apoptosis-resistant state of pulmonary artery smooth muscle cells. The PDK-mediated metabolic switch to aerobic glycolysis is associated with decreased cardiac output and reduced RV contractility. Increased lactate production further impairs RV function secondary to acidosis. Suppression of glucose oxidation reflects the acceptance by the cell of reduced efficiency of ATP generation in exchange for reduced risk of mitochondria-mediated apoptosis and an increased ability to hypertrophy.
overexpression of the serotonin transporter in mice. The same dose of dichloroacetate that is effective in the lung vasculature also decreases PDH phosphorylation, activates PDH, enhances glucose oxidation, and increases contractility in a variety of other rodent PAH models. Dichloroacetate has been used as long-term experimental therapy in adults with glioblastoma multiforme and in children with inherited mitochondrial diseases and lactic acidosis. Dichloroacetate is currently the subject of a clinical trial to determine whether it is a safe and tolerated therapy in patients with moderate PAH (http://www.clinicaltrials.gov; NCT 01083524). To date, the main toxicity of dichloroacetate appears to be a dose-dependent, reversible peripheral neuropathy, although it is well tolerated in patients at appropriate doses.

**Fatty Acid Oxidation in PAH**

**Fatty Acid Oxidation in the Pulmonary Vasculature**

Fatty acid oxidation plays a role in the pulmonary vasculature in PAH. Mice deficient in malonyl-coenzyme A decarboxylase have little fatty acid oxidation and are protected against the development of hypoxia-induced pulmonary hypertension. Malonyl-coenzyme A decarboxylase deficiency exerts its beneficial effects by activating the Randle cycle and promoting glucose oxidation.

**Fatty Acid Oxidation in the RV**

In normal rats, the contribution ratio of glucose oxidation, fatty acid oxidation, and glycolysis to cardiac ATP production is 48%/37%/15%. This is evidence of the importance of glucose oxidation in the normal heart. In Fawn hooded rats with RVH and PAH, the ratios change, reflecting an increased reliance on glycolysis (37%/39%/24%). Dichloroacetate increases the contribution of glucose oxidation to ATP production at the expense of fatty acid oxidation (70%/15%/15%), an illustration of the Randle cycle mechanism.

There appear to be differences in fatty acid oxidation among different models of RVH, with increases being reported in the pulmonary artery banding model versus decreases in Fawn hooded rats. Limited data are available on the oxidative metabolism of fatty acids in human PAH. Acetate is rapidly metabolized into acetyl-CoA and enters into the Krebs cycle. Consequently, 11C-acetate uptake on PET scans can measure net oxidative metabolism in vivo. C-acetate PET was performed in 27 patients with World Health Organization functional class II/III PAH and 9 healthy individuals. The RV oxidative metabolic rate was increased in PAH patients relative to control subjects, although no intervention was performed to assess whether this change was beneficial or maladaptive, nor was the relative contribute of fatty acid versus glucose oxidation determined.
Partially inhibiting fatty acid oxidation appears to be beneficial in RVH models in which fatty acid oxidation is increased. This can be achieved with ranolazine and trimetazidine (Figure 2). These partial inhibitors of fatty acid oxidation are approved for use in patients with angina (United States) and failure of the left side of the heart (Europe), respectively. Inhibition of fatty acid oxidation in RVH increases glucose oxidation and RV ATP levels. In rats with pulmonary artery banding–induced RVH, inhibition of fatty acid oxidation increases exercise tolerance and cardiac output and improves cardiac repolarization, evident clinically by normalization of the QT interval on the surface ECG. The potential therapeutic benefit of trimetazidine has also been observed in monocrotaline-induced RVH. Trimetazidine reduces the creation of free oxygen radicals, increases oxygen consumption, and improves mitochondrial function in cardiac myocytes. Glutaminolysis is accompanied by increased RV expression of mitochondrial malic enzyme and the glutamine transporters SLC1A5 and SLC7A5. Glutaminolysis appears to be induced by ischemic activation of the cMyc transcriptional pathway. Preliminary evidence suggests that glutaminolysis may provide a therapeutic target. In vivo, chronic glutamine antagonism with 6-Diazo-5-oxo-l-norleucine (Figure 3) increases cardiac output, reduces RVH, restores PDH activity, and increases glucose oxidation. However, there are limited preclinical data supporting this strategy in RVH and PAH, and the attempt to exploit this pathway as a cancer therapy was confounded by toxicity. It has yet to be assessed whether glutaminolysis is also induced in the hypertensive pulmonary vasculature.

**Adaptive Versus Maladaptive RVH**

There is increasing recognition of heterogeneity in RVH, with some forms being well tolerated (adaptive RVH) and other forms rapidly resulting in RV failure (maladaptive RVH). PAH patients with adaptive RVH remain stable for many years, whereas those with maladaptive RVH rapidly decompenstate despite a similar RV mass and similar increases in RV pressure. In adaptive RVH, cardiac output remains relatively normal, as do RV ejection fraction and exercise capacity. In maladaptive RVH, cardiac output falls significantly, as do RV ejection fraction and exercise capacity. Maladaptive RVH and RV failure are much more common in scleroderma-associated...
PAH9,70 than in PAH associated with congenital heart disease (ie, the Eisenmenger syndrome7), which is often adaptive. Similarly, in isolated RV, pressure overload caused by pulmonic stenosis, adaptive RVH, characterized by concentric hypertrophy and minimal fibrosis, is the norm. This adaptive RVH is associated with preserved contractility.72,73

The determinants of progression to RV failure in PAH are poorly understood. In carefully controlled rodent models with identical RV mass and RVH severity, there are dramatic differences in cardiac output and likelihood of progression to failure. Adaptive RVH is evident in models of pulmonary artery banding, whereas maladaptive RVH occurs in PAH models induced by monocrotaline or the combination of chronic hypoxia plus the VEGF-2 receptor antagonist SU5416.42

There is greater aerobic glycolysis along with PDH inhibition in maladaptive monocrotaline RVH than in adaptive pulmonary artery banding–RVH.2

There appears to be a transition point at which RVH changes from being adaptive to maladaptive.44 The transition to maladaptive RVH is associated with a decrease in angiogenesis, inhibition of HIF-1α in the RV, and a decrease in glucose uptake.45 In maladaptive RVH, there is also chamber-specific dysregulation of the autonomic nervous system with desensitization and downregulation of α-, β-, and dopaminergic receptors in the RV.74 In maladaptive RVH, many of these changes extend into the left ventricle.74 Factors that determine whether RVH will be adaptive or maladaptive include the presence and severity of RV ischemia, autonomic dysregulation, fibrosis, angiogenesis, and metabolic changes.

The combination of ischemia, metabolic abnormalities, and impaired contractility suggests that the hypokinetic RV may be a form of myocardial hibernation.50 Supporting this argument, successful lung transplantation for PAH usually results in reversal of RV dysfunction.75 Similarly, in chronic thromboembolic pulmonary hypertension, the function of the RV typically returns to normal within weeks after pulmonary endarterectomy.76

Controversies

Controversy remains as to whether HIF-1α prevents or promotes failure of the RV in PAH. Sutendra et al44 compared the initially adaptive hypertrophy seen in rats with monocrotaline-induced PAH with rats that were later in their disease course and had developed signs of heart failure. The compensated RV had low production of mitochondria-derived ROS and increased expression of HIF-1α with evidence of activation of its downstream pathway (increased expression of Glut1, VEGF, and stromal-derived factor 1). As a result of HIF-1α activation, there were increased angiogenesis and increased 18FDG uptake on PET. The transition to decompensated RVH was marked by a sharp rise in mitochondria-derived ROS and an associated inhibition of HIF-1α and activation of p53, both of which contributed to downregulation of PDK and decreased glucose uptake. The authors found that decoupling was associated with a decrease in angiogenic factors and angiogenesis.44 This finding is consistent with the work of others who have noted capillary rarefaction in maladaptive RVH.20,42 However, the conclusion that this decrease in RV angiogenesis reflects loss of HIF-1α44 differs from that of Drake et al,77 who noted preserved HIF-1α expression in severe RVH and attributed impaired angiogenesis to failure of downstream angiogenic signaling, evident as reduced VEGF and Akt expression.

The debate about whether activation of the glycolytic pathways is adaptive or maladaptive is also informed by the cancer literature. In malignancy, excessive activation of oncogenes such as cMyc results in a lethal oncogenic stress response that is caused by enhanced aerobic glycolysis and glutaminolysis.78 Likewise, in the failing RV in PAH, there appears to be more activation of PDK4 and greater upregulation of Glut1 than in a compensated RV.79 This would suggest that ongoing or excessive aerobic glycolysis might be expected to be maladaptive.

There is also debate about the predominant transcriptional pathways activated in RVH. Although some groups report that HIF-1α is the predominant transcription factor in RVH, we have observed that much of the transcriptional basis for metabolic remodeling in the RV results from activation of cMyc21 and Forkhead box protein O1.79 In contrast, HIF-1α appears to be the predominant transcription factor governing the metabolic shift to aerobic glycolysis in the lung vasculature.10,31

Metabolism may also be abnormal in the left ventricle in PAH. Myocardial metabolism in the interventricular septum, as measured with the labeled fatty acid β-methyl-p-13I-iodophenyl-pentadecanoic acid (BMIPP), is reduced in PAH patients.79 The impairment of septal BMIPP uptake is proportional to the degree of pulmonary hypertension. The coronary flow reserve of the left ventricle is also impaired in patients with PAH.79 The role of metabolic changes in the left ventricle merits further investigation.

The prediction of whether a metabolic change in the heart will be adaptive or maladaptive is likely contextual, depending on the disease, species, and time course of the change.80 For example, a mouse model of diabetic cardiomyopathy, created by transgenic overexpression of PDK4, would have been predicted to be deleterious. However, sustained activation of PDK4 led to transcriptional and posttranscriptional changes in metabolism that adapted the heart to the observed suppression of glucose oxidation resulting in persistence of high rates of fatty acid oxidation.81 PDK4-overexpressing mice had increased cardiac levels of AMP-activated protein kinase and its target, peroxisome proliferator-activated receptor-γ coactivator1-α.

Not all metabolic derangements require a metabolic solution. For example, increased RV 18FDG uptake in PAH patients is reduced by long-term therapy with intravenous epoprostenol, a vasodilator prostaglandin55 (Figure 7C and 7D). Similarly, lung 18FDG uptake in monocrotaline rats is reduced by imatinib, a tyrosine kinase inhibitor.31 In both cases, this suggests that reducing the pressure overload and shear stress in PAH may turn off an ongoing metabolic program. An additional strategy to indirectly correct metabolic abnormalities in PAH would be to reduce RV ischemia with β-blockers. Indeed, capillary rarefaction in the RV of rats with PAH is reversible with β-blockers.82 β-Blockers are currently being studied in clinical trials in PAH patients (http://www.clinicaltrials.gov; NCT 01246037) and are reported to be well tolerated.83
Limitations
Although this review focuses on metabolism, many other factors determine the success of the response of the RV to pressure and volume overload, notably the occurrence of fibrosis. RV fibrosis is an important predictor of maladaptive physiology in both the lung and RV. In PAH patients, late gadolinium enhancement at the RV insertion point into the septum is indicative of fibrosis. Late gadolinium enhancement is associated with RV dilation and reduced RV ejection fraction and predicts time to clinical worsening.61 The accumulation of collagen with a resulting loss of RV compliance is also seen in the chronic hypoxia plus SU5416 rat model of PAH.60 This maladaptive response may also present itself as a therapeutic target and in rodents can be prevented by angiotensin-converting enzyme inhibitors such as enalapril.62 The importance of mitochondrial metabolic abnormalities in promoting fibrosis is an area that requires further research.

Conclusions
Metabolic abnormalities are observed in the RV and pulmonary circulation in PAH in both preclinical models and patients. Therapies that promote glucose oxidation or inhibit fatty acid oxidation or glutaminolysis may represent new therapeutic targets in PAH. These therapies would be predicted to have benefits on both the RV and pulmonary vasculature. The potential benefit of metabolic therapies for shared abnormalities in the cardiopulmonary unit suggests a new and attractive therapeutic paradigm in PAH. However, carefully designed clinical trials are required to assess the safety and therapeutic value of metabolic therapies.

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Disclosures
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References


34. Ryan and Archer PAH: Disordered Mitochondrial Metabolism/Dynamics 1701


Emerging Concepts in the Molecular Basis of Pulmonary Arterial Hypertension: Part I: Metabolic Plasticity and Mitochondrial Dynamics in the Pulmonary Circulation and Right Ventricle in Pulmonary Arterial Hypertension

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