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Emerging concepts in the molecular basis of pulmonary arterial hypertension (PAH): Part I: Metabolic plasticity and mitochondrial dynamics in the pulmonary circulation and right ventricle in PAH

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Introduction

Mitochondria are central to cellular metabolism. The mitochondria's metabolic pathways include fatty acid oxidation, glucose oxidation and glutaminolysis. The initial step in glucose metabolism occurs in the cytosol, where glycolysis converts glucose to pyruvate[1] (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)[3]. Under pathologic 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, the organism's developmental stage, and pathologic 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 crosstalk 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[3]. Another example of

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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[5]. Warburg noted that this shift to glycolysis contributed to the growth and survival advantage of cancer cells[5]. He also observed, but could not explain, accumulation of ammonia in his cancer tissue culture. Ultimately this proved to relate to a concomitant upregulation of glutaminolysis in cancer cells. Aerobic glycolysis results in a reliance on glycolysis to produce ATP despite the presence of sufficient oxygen to have allowed pyruvate generation and mitochondrial glucose oxidation. Aerobic glycolysis usually reflects active inhibition of one or more mitochondrial enzymes, notably inhibition of PDH by pyruvate dehydrogenase kinases (PDK). These acquired changes in metabolism alter the cell's bioenergetics status, susceptibility to hypertrophy and fibrosis, rates of proliferation and apoptosis, angiogenesis and contractility. Importantly, the cell's metabolic choices can be pharmacologically manipulated, offering the potential for metabolic therapies.

In addition to generating adenosine triphosphate (ATP), mitochondria are constantly dividing and joining together[6]. These highly conserved and regulated processes are called fission and fusion, respectively[7]. These non-canonical mitochondrial functions (fission, fusion), as well as migration, are called *mitochondrial dynamics*.[8] Mitochondrial dynamics are important in physiology, participating in oxygen sensing[9] and the distribution of mitochondria to daughter cells during mitosis[10]. 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[7]. 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.

Mitochondria and metabolism in PAH

Vascular cells and right ventricular 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, due to pathologic activation of transcription factors such as cMyc, Forkhead transcription factor (FOXO1) and hypoxia inducible factor (HIF-1α), and PDK-induced PDH inhibition. In the hypertrophied right ventricle, cancer-like metabolic changes, aerobic glycolysis and glutaminolysis, reduce energy production and contractility[1]. PAH and cancer cells also share a mitochondrial morphologic phenotype (increased mitochondrial fragmentation) that is due to a fission/fusion imbalance.[11] 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 right ventricle, to improve contractility, increase cardiac

output and reduce hypertrophy.[10,12] In this review, we will summarize the mechanism of several mitochondrial abnormalities in PAH and discuss potential therapeutic targets in the pulmonary vasculature and right ventricle.[13] Readers are referred to several recent reviews on the subject of metabolism[13–16] and mitochondrial dynamics[7] 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[17]. In the adult heart, the predominant energy source is fatty acid oxidation (60–90%); however, glucose metabolism continues to contribute to ATP production[18]. Although classically considered a secondary source of ATP production in the adult heart[19], direct measurement shows that glucose oxidation remains an important source of ATP in the normal right ventricle, accounting for 48% of total ATP produced[20].

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 (FATP 1 and 6) and the solute carrier proteins (SLC 1A5 and 7A5), respectively (Fig 1–3). When substrate utilization is increased, transporter expression rises, as is the case for Glut and SLC1A5 in the hypertrophied right ventricle in PAH[20, 21]. Second, each of the pathways drives 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[21], although independent corroboration is required. Fourth, it appears that most of the metabolic changes seen in the right ventricle 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 animal's hemodynamic and functional state.

Despite these commonalities the pathways vary greatly in their bioenergetic yield. A fatty acid containing 6 carbons subjected to beta-oxidation in the mitochondria can yield 48 ATP. However, fatty acid oxidation comes at a price in that it uses approximately 10% more oxygen than glucose oxidation to generate the same amount of ATP[22] (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 a excess production of lactate. In aerobic glycolysis only 2 ATP are generated per mole of glucose and lactate is produced[2].

Metabolism of the amino acid glutamine via glutaminolysis, also occurs in PAH and cancer. In glutaminolysis, glutamine is hydrolysed to glutamate and converted to α-ketoglutarate in the mitochondria. α-ketoglutarate then enters Krebs' cycle and replenishes metabolic intermediates, thereby supporting rapid cell growth. In addition, glutaminolysis can increase nitrogen anabolism which further supports cell growth[23]. Glutaminolysis was originally identified in cancer cells[23], but has recently been found to be induced in the heart during RVH (Figure 3)[21]. Glutaminolysis in the RV generates modest energy but rewards cells with amino acid intermediates that permit rapid cell growth[21].

A Brief Review of Mitochondrial Dynamics

Mitochondrial fusion is mediated by large GTPases, mitofusin-1 and mitofusin-2[12], which reside in the outer mitochondrial membrane, and a GTPase called optic atrophy-1, in the inner mitochondria membrane[7]. Fission is mediated by the GTPase, dynamin-related protein 1 (DRP1)[10], which upon activation moves from the cytosol to the outer mitochondrial membrane. There it interacts with non-GTPase binding partners, such as mitochondrial fragmentation factor (MFF) and fission factor 1 (Fis1), resulting in multimerization and division of the mitochondria (reviewed in[7]).

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 DRP1, such as mdivi-1, regress 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 co-activator, peroxisome proliferator-activated receptor γ coactivator 1-α[12]. Augmentation of mitofusin-2 expression is antiproliferative, 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 where 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 *pseudo-hypoxia* 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 pseudo-hypoxic state is associated with impairment of a well-established mitochondria-ROS-HIF-1α-Kv1.5 oxygen-sensing pathway[26]. In PAH,

downregulation of the mitochondrial hydrogen peroxide generating enzyme superoxide dismutase 2 (SOD2) decreases production of the redox signaling molecule, hydrogen peroxide, 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 production of reactive oxygen species (ROS) by the mitochondrial electron transport chain. Loss of physiologic levels of ROS inhibit and downregulate 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 PO_2 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 either at 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, 29]. SOD2 is a nuclear-encoded, mitochondrial enzyme responsible for the production of the diffusible, redox signaling molecule hydrogen peroxide (H_2O_2)[26, 27, 30]. Loss of SOD2-mediated production of hydrogen peroxide activates HIF-1α[10, 27]. The relationship between SOD2 and HIF-1α is robust. Simply downregulating SOD2 in normal pulmonary artery smooth muscle cells using small inhibitory RNA, activates HIF-1α despite normal PO_2. Likewise, once HIF-1α is activated there is a clear phenotypic shift in pulmonary artery smooth muscle cells, which become hyperproliferative[31] 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 persist in culture, despite abundant O_2[27].

Transcriptional repression of SOD2 expression occurs through methylation of two 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 hydrogen peroxide 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 the Fawn-hooded rat but results from a homozygous mutation in the von Hippel-Lindau gene (VHL 598C to T) that removes the ubiquitination signal for HIF-1α degradation[35] and thus impairs proteosomal 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 2–3 hours. Interestingly, cobalt does not cause cell proliferation in culture, likely due to nonspecific toxicity. However, chronic, in vivo administration of cobalt engenders pulmonary hypertension and right ventricular hypertrophy 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 chemotin, 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[36, 37].

Metabolic remodeling in the hypertrophied right ventricle in PAH

Altered right ventricular metabolism in PAH is transcriptionally mediated. However, in the right ventricle the likely precipitant is ischemia, rather than impaired oxygen sensing. In RVH there is ischemia[38] and decreased coronary flow reserve[39]; 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 right ventricle systolic pressure is low relative to the driving pressure in the aorta. In right ventricle pressure overload, the systolic perfusion gradient between the aortic and right ventricle systolic pressures may disappear limiting right coronary artery flow to diastole. This essentially halves the amount of blood being supplied to the hypertrophied right ventricle, which has increased metabolic demands[40]. At extremes of pulmonary hypertension, when right coronary artery perfusion pressure falls below 50 mmHg, right ventricle contractile function declines[41]. Right ventricular 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 angiopoeitin-1 (Figure 5B)[21]. Occlusive microvascular disease and capillary rarefaction has been seen in the right ventricle in animal models of maladaptive PAH[20, 42] and in patients with scleroderma-associated PAH (Figure 5C)[21].

The role played by HIF-1α in the metabolic remodeling of the right ventricle in PAH is less clear than its role in the pulmonary vasculature. Several investigators find HIF-1α is

increased in rodent RVH models[43, 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 HIF-1α's temporal profile (reviewed in the section on controversies).

A central role for inhibition of pyruvate dehydrogenase 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 Krebs' cycle is limited and energy production is reduced[45] (Figure 1).

The four PDK isoforms differ in their transcriptional regulation and tissue distribution[46]. PDK2 appears to be the predominant human isoform in many tissues[46], however the magnitude and consequences of regional isoform heterogeneity amongst tissues is not adequately studied. For example, PDK1 expression is transcriptionally upregulated by HIF-1α[28, 47], whereas PDK4, which lacks a hypoxia recognition element in its promoter and is thus not directly regulated by HIF-1α is induced by FOXO1[20, 48]. However, HIF-1α can transcriptionally upregulate estrogen-related receptor γ (ERRγ), which is capable of transcriptionally increasing PDK4 expression[49].

The dominant PDK isoforms in a specific tissue can change with disease and this pathologic isoform variation is largely unstudied. For example in rodent PAH the dominant PDK isoforms upregulated in the right ventricle are PDK 2 and 4[20]. Anecdotal evidence suggests PDK4 is also upregulated in the human right ventricle[50]. In the lung the predominant isoforms upregulated in PAH are PDK1 and PDK2[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 whilst in others PDK is active under physiologic condition. In skeletal muscle, tonic activation of PDK2 contributes to regulation of carbohydrate oxidation and production of reducing equivalents for the electron transport chain[47]. In contrast, there appears to be little tonic PDK activity in the normal right ventricle and pulmonary artery, as indicated by the absence of effect of a pan-PDK inhibitor, dichloroacetate (Figure 1), on metabolism and the cellular electrophysiology of normal right ventricular and pulmonary vascular cells[2, 51–53].

When the mitochondrial PDH complex is active, pyruvate is converted to acetyl coenzyme A, which fuels Krebs' cycle, generating electron donors for the electron transport chain and fueling generation of ATP[19]. However, in PAH PDH inhibition inhibits the electron transport chain and increases reliance on aerobic glycolysis[13, 44]. This metabolic shift contributes to the hyperproliferative, apoptosis-resistant state of pulmonary artery smooth muscle cells[53]. The PDK-mediated metabolic switch to aerobic glycolysis is associated with decreased cardiac output and reduced right ventricle contractility[20, 2]. Increased lactate production further impairs right ventricle function secondary to acidosis. Suppression of glucose oxidation reflects the cell's acceptance of reduced efficiency of ATP generation in

exchange for reduced risk of mitochondrial-mediated apoptosis and an increased ability to hypertrophy[1].

Increased glycolysis is associated with increased glucose flux, allowing right ventricular glycolysis to be detected on cardiac ^{18}F-fluorodeoxyglucose-positron emission tomography (^{18}FDG-PET) scans in patients and in animal models (Figure 6)[31, 54]. There is also preliminary evidence that reduction of right ventricle afterload by initiation of pulmonary vasodilators reduces right ventricular uptake of ^{18}FDG in patients (Figure 7)[55]. In a case report comparing a PAH patient who died from rapidly decompensating right ventricular failure with a long-term survivor, expression of both PDK4 and the Glut-1 transporter were 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[55]. The dichloroacetate binding pocket is relatively small (volume = 211 Å^3)[57] and it is buried within the PDK structure, making it relatively inaccessible to molecules larger than pyruvate[26]. 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 Å^3), more accessible ATP-binding pocket[57]. Dichloroacetate-induced metabolic changes depolarize mitochondria and induce apoptosis while inhibiting pulmonary artery smooth muscle cell proliferation[26]. Dichloroacetate is relatively specific for abnormal tissues and has little effect on normal cardiac or vascular cells, in which PDK isoforms are inactive[51, 52]. Oral dichloroacetate is effective in regressing pulmonary vascular disease and improving functions in preclinical models of pulmonary hypertension, including chronic hypoxic pulmonary hypertension[52, 53], monocrotaline-PAH[52], spontaneous PAH in Fawn-hooded rats[20] and PAH induced by transgenic overexpression of the serotonin transporter in mice[51]. 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[2, 58]. Dichloroacetate has been used as chronic experimental therapy in adults with glioblastoma multiforme[59] and in children with inherited mitochondrial diseases and lactic acidosis[60]. Dichloroacetate is currently the subject of a clinical trial to determine if it is a safe and tolerated therapy in patients with moderate PAH (NCT 01083524). To date dichloroacetate's main toxicity appears to be a dose dependent, reversible peripheral neuropathy[61, 62], 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 from developing hypoxia-induced pulmonary hypertension[63]. Malonyl-coenzyme A decarboxylase deficiency exerts its beneficial effects by activating the Randle cycle and promoting glucose oxidation.

Fatty Acid Oxidation in the Right Ventricle

In normal rats, the contribution ratio of glucose oxidation, fatty acid oxidation and glycolysis to cardiac ATP production is 48 %/37%/15%, respectively[20]. 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%)[20]. 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[20].

There appear to be differences in fatty acid oxidation amongst different models of RVH, with increases being reported in the pulmonary artery banding model[4] versus decreases in Fawn-hooded rats[20]. There are limited data available regarding oxidative metabolism of fatty acids in human PAH. Acetate is rapidly metabolized into acetyl-CoA and enters into Krebs' cycle. Consequently, ^{11}C-acetate uptake on PET scans can measure net oxidative metabolism *in vivo*. ^{11}C-acetate PET was performed in 27 patients with WHO functional class II/III PAH and 9 healthy individuals[64]. The RV oxidative metabolic rate was increased in PAH patients relative to controls, 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 beneficial in RVH models in which fatty acid oxidation is increased. This can be achieved using ranolazine and trimetazidine (Figure 2). These partial inhibitors of fatty acid oxidation are approved for use in patients with angina (USA) and left heart failure (Europe), respectively. Inhibition of fatty acid oxidation in RVH increases glucose oxidation and right ventricular ATP levels[4]. In rats with pulmonary artery banding-induced RVH, inhibition of fatty acid oxidation increases exercise tolerance, cardiac output and improves cardiac repolarization, evident clinically by normalization of the QT interval on the surface electrocardiogram[4]. The potential therapeutic benefit of trimetazidine has also been observed in monocrotaline-induced RVH[65]. Trimetazidine reduces the creation of free oxygen radicals, increases oxygen consumption, and improves mitochondrial function in cardiac myocytes[66]. ^{18}FDG-PET studies have demonstrated that inhibition of fatty acid oxidation with trimetazidine in dilated cardiomyopathy results in a corresponding increase in glucose oxidation[67]. Ranolazine is now being studied in PAH patients in Phase 1 clinical trials (NCT01757808 and NCT01174173).

Glutaminolysis in PAH

There is little if any glutaminolysis in the normal heart. However in RVH, glutaminolysis is selectively induced in the right ventricle[21]. Increased glutaminolysis in monocrotaline-RVH is accompanied by increased right ventricle expression of mitochondrial malic enzyme and the glutamine transporters, SLC1A5 and SLC7A5[21]. Glutaminolysis appears to be induced by ischemic activation of the cMyc transcriptional pathway[21]. Preliminary evidence suggests that glutaminolysis may provide a therapeutic target. I*n vivo*, chronic glutamine antagonism with 6-Diazo-5-oxo-L-norleucine (DON) (Figure 3), increases cardiac output, reduces RVH, restores PDH activity and increases glucose oxidation[21]. However, there is

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[68]. 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 right ventricle failure (maladaptive RVH). PAH patients with adaptive RVH remain stable for many years, whereas others, with maladaptive RVH, rapidly decompensate despite similar right ventricular mass and similar increases in right ventricle pressure[50]. In adaptive RVH, cardiac output remains relatively normal, as does right ventricular ejection fraction and exercise capacity. In maladaptive RVH, cardiac output falls significantly, as does right ventricular ejection fraction and exercise capacity. Maladaptive RVH and right ventricular failure is much more common in scleroderma-associated PAH[69, 70], than in PAH associated with congenital heart disease (i.e. Eisenmenger's syndrome[71]), which is often adaptive. Similarly, in isolated right ventricle pressure overload due to pulmonic stenosis, adaptive RVH, characterized by concentric hypertrophy and minimal fibrosis, is common and is associated with preserved contractility[72, 73].

The determinants of progression to right ventricle failure in PAH are poorly understood. In carefully controlled rodent models with identical right ventricular 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 and 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 right ventricle, 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 right ventricle[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 right ventricular ischemia, autonomic dysregulation, fibrosis, angiogenesis, and metabolic changes.

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

Controversies

There remains controversy as to whether HIF-1α prevents or promotes failure of the right ventricle in PAH. Sutendra compared the initially adaptive hypertrophy seen in rats with monocrotaline-induced PAH to rats who were later in their disease course and had developed signs of heart failure. The compensated right ventricle had low production of mitochondria-derived reactive oxygen species (mROS) and increased expression of HIF-1α with evidence of activation of its downstream pathway (increased expression of Glut1, VEGF, and stromal-derived factor 1)[44]. As a result of HIF-1α activation there was increased angiogenesis and increased ^{18}F-fluorodeoxyglucose uptake on PET. The transition to decompensated RVH was marked by a sharp rise in mROS and an associated inhibition of HIF-1α, and activation of p53, both of which contributed to down-regulation of PDK and decreased glucose uptake. The authors found that decompensation was associated with a decrease in angiogenic factors and angiogenesis[44]. This latter 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-1α[44] differs from that of Drake et al, 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[77].

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 right ventricle in PAH there appears to be more activation of PDK4 and greater upregulation of Glut1 than in a compensated right ventricle[50]. 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 right ventricle results from activation of cMyc[21] and FOXO1[19]. 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 using the labeled fatty acid, β-methyl-p-^{123}I-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[39]. The role of metabolic changes in the left ventricle merits further investigation.

Prediction whether a metabolic change in the heart will be adaptive or maladaptive is likely contextual, depending on the disease and 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, chronic activation of

PDK4 led to transcriptional and post-transcriptional changes in metabolism that adapted the heart to cope with the observed suppression of glucose oxidation and the resulting chronic 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 coactivator-1.

Not all metabolic derangements require a metabolic solution. For example, increased right ventricular ^{18}F-fluorodeoxyglucose uptake in PAH patients is reduced by long term therapy using intravenous epoprostenol, a vasodilator prostaglandin[55] (Figure 7C–D). Similarly, lung ^{18}F-fluorodeoxyglucose 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 right ventricle ischemia using β-blockers. Indeed, capillary rarefaction in the right ventricle of rats with PAH is reversible with β-blockers[82]. β-blockers are currently being studied in clinical trials in PAH patients (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 right ventricle's response to pressure and volume overload, notably the occurrence of fibrosis. Right ventricle fibrosis is an important predictor of maladaptive physiology, both in the lung and right ventricle. In PAH patients, late gadolinium enhancement at the RV insertion point into the septum is indicative of fibrosis. Late gadolinium enhancement is associated with right ventricle dilation, reduced right ventricle ejection fraction, and predicts time to clinical worsening[61]. The accumulation of collagen with a resulting loss of right ventricle compliance is also seen in the chronic hypoxia plus SU5416 rat model of PAH[80]. This maladaptive response can also present itself as a therapeutic target, and in rodents can be prevented by inhibitors of the angiotensin-converting enzyme, such as enalapril[62]. The importance of mitochondrial metabolic abnormalities in promoting fibrosis is an area of where research is required.

Conclusions

Metabolic abnormalities are observed in the right ventricle and pulmonary circulation in PAH, both in preclinical models and in 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 both on the right ventricle 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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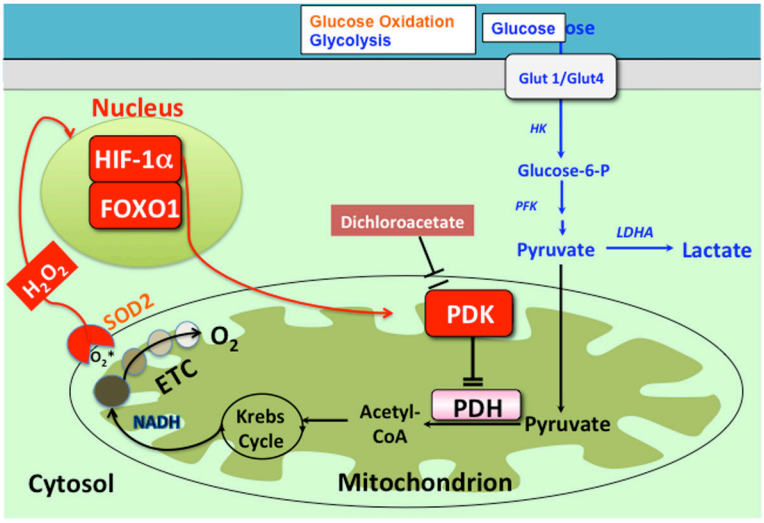

Figure 1.

Mechanism of impaired glucose oxidation and enhanced aerobic glycolysis in PAH. Changes in redox signaling, such as downregulation of SOD2 and the resultant decrease in H_2O_2 signaling, can activate transcription factors (i.e. HIF-1α) which in turn upregulate PDK. PDK inhibits PDH, which impairs oxidative glucose metabolism, causing the cell to rely on other forms of metabolism, such as aerobic glycolysis. The small molecular inhibitor of PDK, dichloroacetate, can reactivate PDH and restore oxidative glucose metabolism. Abbreviations: ETC = electron transport chain, FOXO1 = Forkhead box protein O1, HK = hexokinase, HIF-1α= Hypoxia inducible factor 1α, H_2O_2 = hydrogen peroxide, LDHA = lactate dehydrogenase A, PDH = Pyruvate dehydrogenase, PDK = Pyruvate dehydrogenase kinase, PFK = phosphofructokinase. Reprinted with permission from [2].

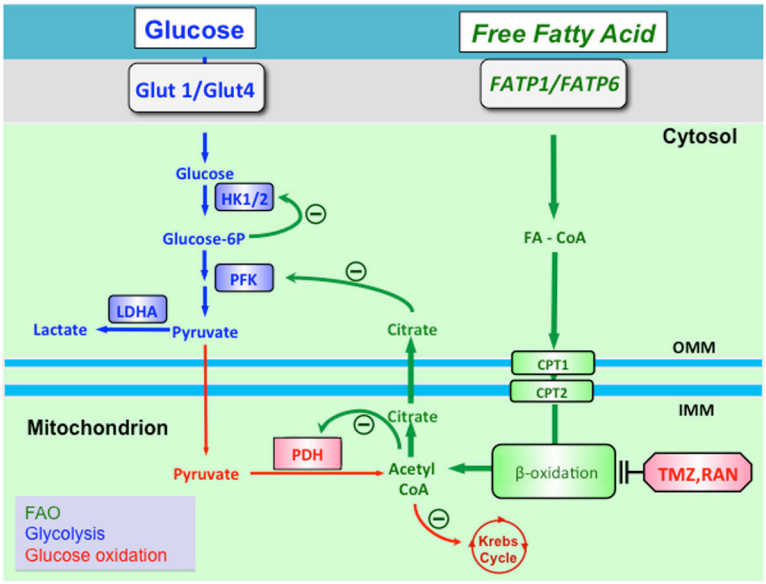

Figure 2.

Manipulating fatty acid and glucose oxidation in PAH: The Randle's cycle. Randle's 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 inhibits PDH (in the mitochondria) and phosphofructokinase (in the cytosol). This feedback (and other indicated feedback mechanisms) slow glucose oxidation under conditions where there is substantial fatty acid oxidation. The pharmacologic inhibitors of fatty acid oxidation, trimetazidine and ranolazine, can restore glucose oxidation by partially inhibiting fatty acid oxidation and activating Randle's cycle. Abbreviations: CPT1/2 = Carnitine palmitoyltransferase 1/2, FA-CoA = fatty acyl-CoA, FATP1/6 = Fatty acid transport protein 1/6, Glut1/4 = Glucose transporter ¼, HK = hexokinase, HIF-1α= Hypoxia inducible factor 1α, LDHA = lactate dehydrogenase A, PDH = Pyruvate dehydrogenase, PDK = Pyruvate dehydrogenase kinase, PFK = phosphofructokinase, OMM=outer mitochondrial membrane, IMM=inner mitochondrial membrane, TMZ=trimetazidine, RAN=ranolazine. Reprinted with permission from [4].

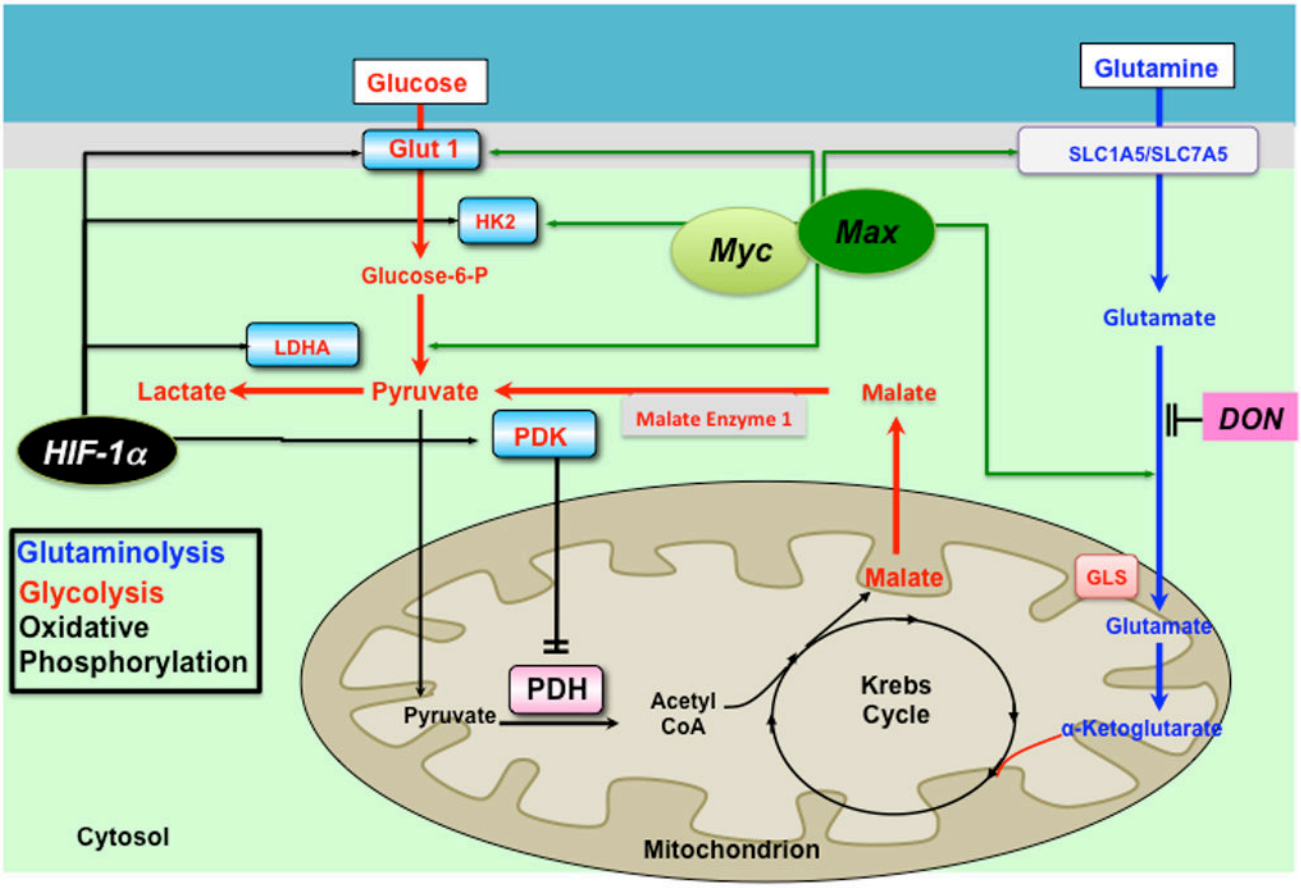

Figure 3.

Proposed mechanism of glutaminolysis in the hypertrophied right ventricle. 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 Krebs' cycle leading to production of malate. 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. DON can inhibit glutaminolysis and restore glucose oxidation. HIF-1α increases the transcription of the some of the same glycolytic mediators as cMyc and Max, notably Glut1 and HK2. Abbreviations: Glut1 = Glucose transporter 1, HK = hexokinase, HIF-1α= Hypoxia inducible factor 1α, LDHA = lactate dehydrogenase A, PDH = Pyruvate dehydrogenase, PDK = Pyruvate dehydrogenase kinase, PFK = phosphofructokinase. DON= 6-diazo-5-oxo-l-norleucine. Reprinted with permission from [21].

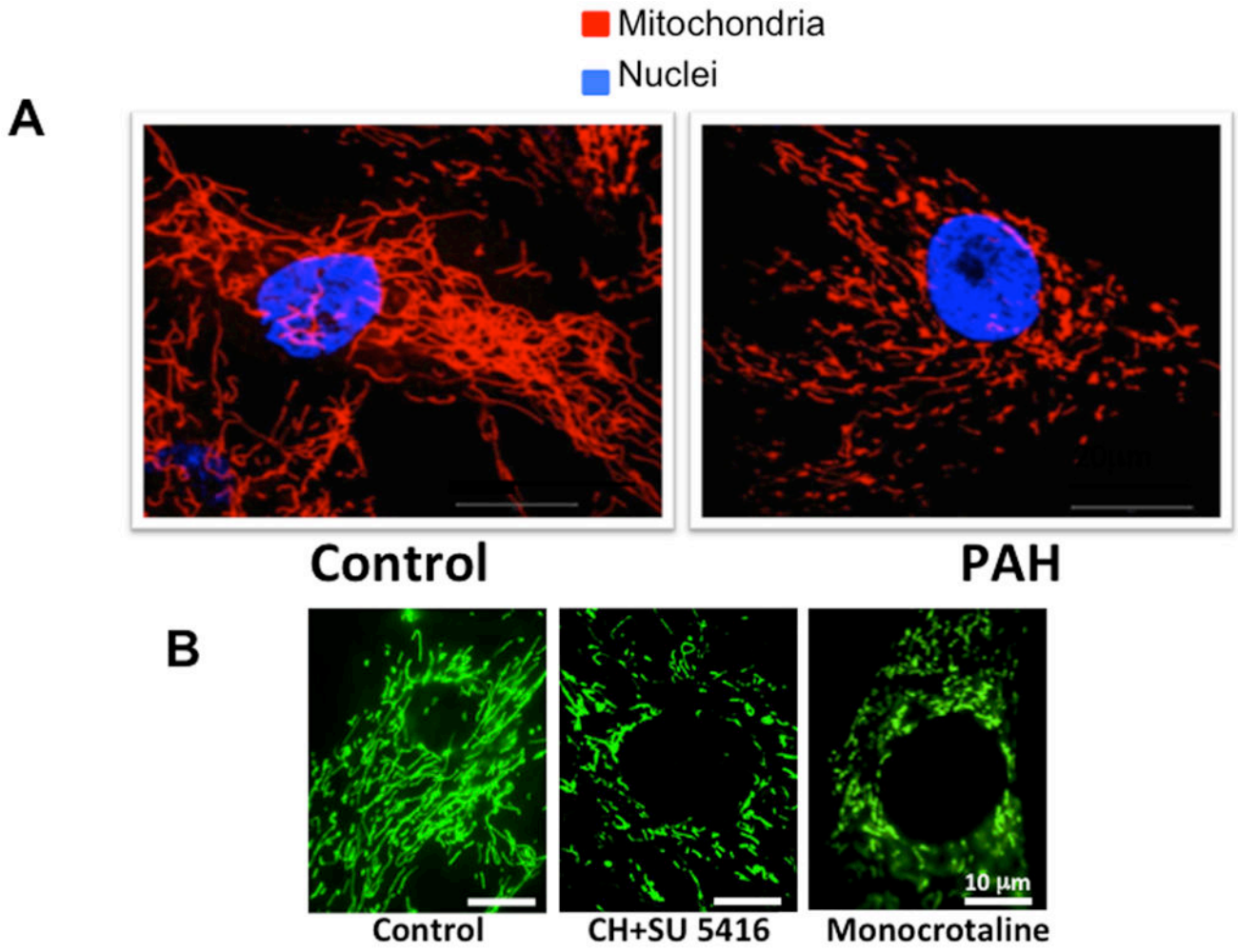

Figure 4.

Mitochondrial fragmentation in Pulmonary Arterial Hypertension (PAH). **A.** Mitochondria are more fragmented in PAH *versus* control pulmonary artery smooth muscle cells (PASMCs). Quantification of the mitochondrial fragmentation count reveals a doubling of the number of individual mitochondria in PAH *versus* control PASMCs. Scale bar = 20 μm. Reprinted with permission from [10]. **B.** Increased mitochondrial fragmentation observed in PASMC of rats with PAH induced by exposure to chronic hypoxia plus the VEGF receptor antagonist, SU5416 (CH+SU 5416) or monocrotaline. Mitochondria were imaged by infection of cells with BacMam virus carrying a mitochondrial-targeted green fluorescent protein transgene. Reprinted with permission from [12].

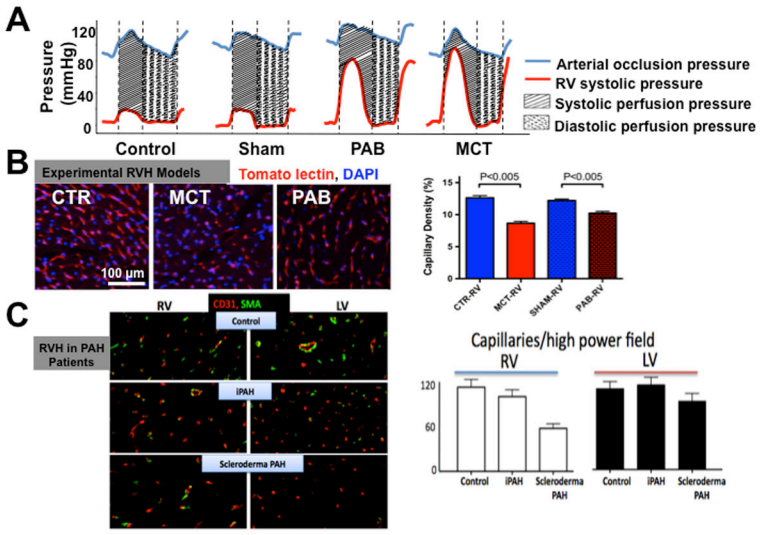

Figure 5.

Adaptive versus Maladaptive Forms of RVH. **A.** Catheterization data shows simultaneous RV pressure and aortic pressure (AoP) in various rat RVH models. Despite similar coronary perfusion pressure (defined as the Δ Pressure Ao-RV), there is worse RV function and exercise capacity in monocrotaline (MCT) *versus* 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 (Monocrotaline-RVH) than in an adaptive form of RVH (created by pulmonary artery banding, PAB). Reprinted with permission from [21]. **C.** Representative images and mean data showing greater right ventricular capillary rarefaction in scleroderma-PAH patients, known for the greater propensity to develop RV failure, than in normal subjects or patients with idiopathic PAH. Reprinted with permission from [21]. Red stain: CD31 (an endothelial cell marker); Green stain (smooth muscle actin).

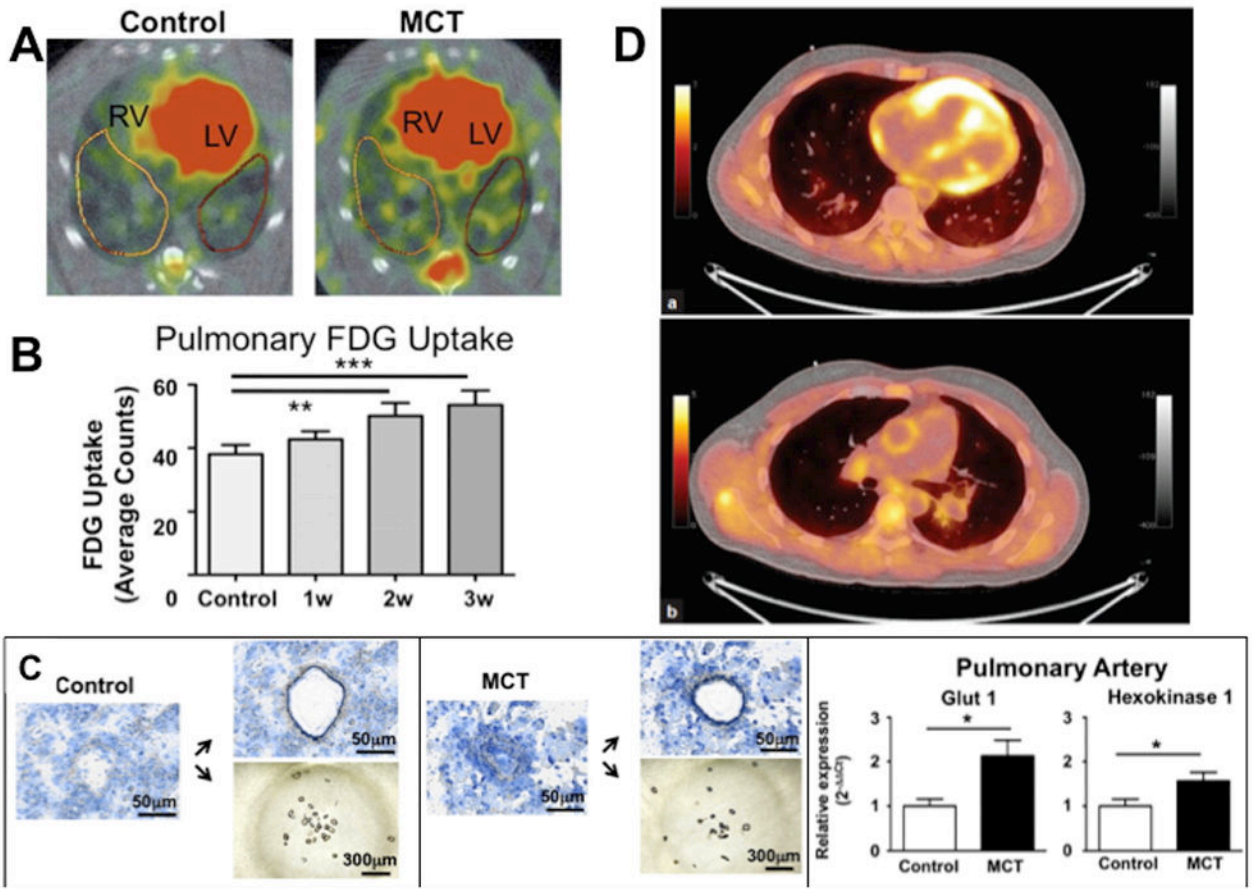

Figure 6.

Detection of enhanced aerobic glycolysis in the right ventricle and small pulmonary arteries. **A.** Increased ^{18}F-fluorodeoxyglucose (FDG) uptake in the right ventricle (RV) and the lung parenchyma of MCT animals. LV=left ventricle. **B.** Quantification of pulmonary ^{18}F-FDG uptake measured with PET. By week 2 after monocrotaline injection, lung ^{18}F-FDG uptake is significantly greater than in control lungs. **C.** Laser capture microdissection (LCM) confirms the vascular origin of the glycolytic signal in the lungs of monocrotaline (MCT) rats. Small pulmonary precapillary resistance vessels (<100 μm in diameter) or pieces of airway tissue were collected by LCM. Reprinted with permission from [31]. **D.** (a) PET and (b) fused PET/CT of the right ventricle and pulmonary trunk of an idiopathic PAH subject showing increased ^{18}F-FDG uptake. Reprinted with permission from [54].

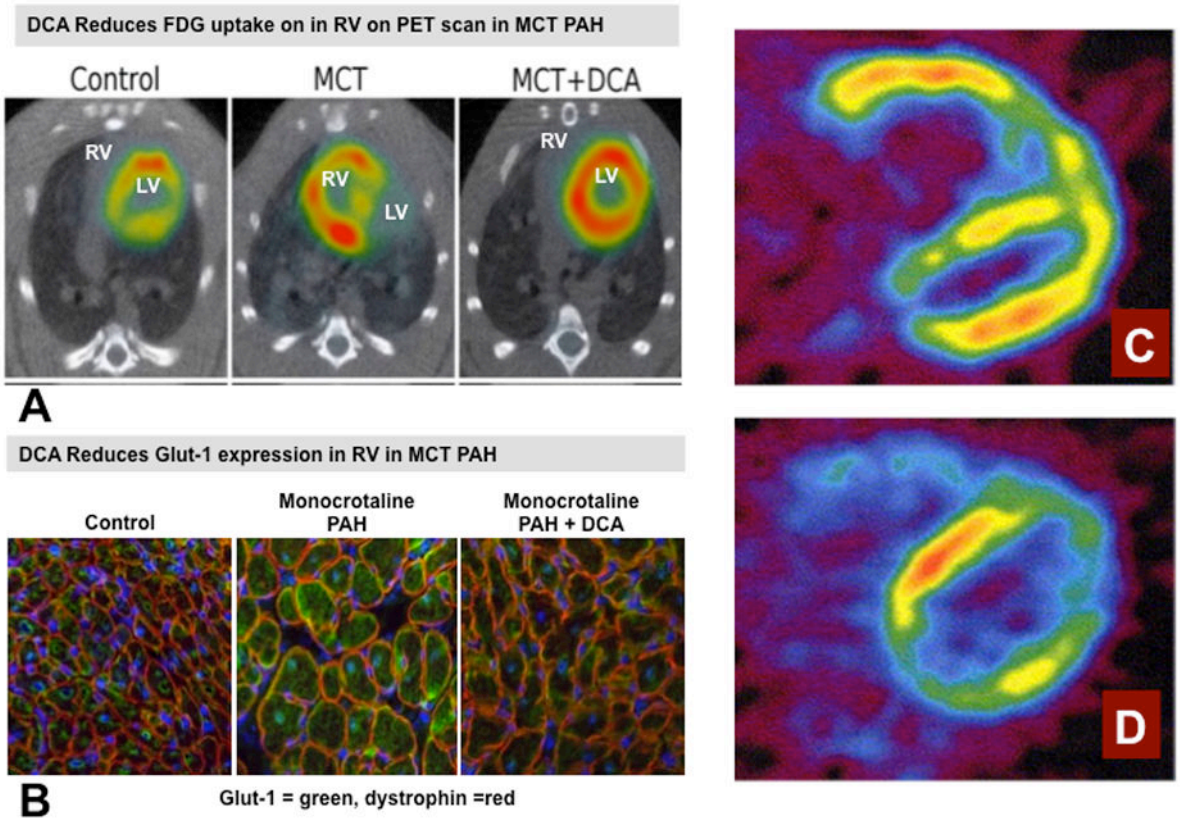

Figure 7.

Reversibility of metabolic changes in the RV in PAH. **A.** Representative images and mean data showing that the increased ^{18}F-fluorodeoxyglucose uptake in the right ventricle (RV) on PET scans in monocrotaline (MCT) rats which is reduced by dichloroacetate (DCA) (See Figure 1). Reprinted with permission from [2]. **B.** Immunostaining showing that the increased Glut1 expression in RV myocytes in monocrotaline-induced RVH is reduced by chronic oral consumption of the PDK inhibitor, dichloroacetate (DCA). The merge of the staining of Glut1 (green) and dystrophin (red) in RV shows that more Glut1 is expressed at the myocyte membrane in RVH. Reprinted with permission from [2]. **C & D.** Representative midventricular transaxial ^{18}F-fluorodeoxyglucose PET images of a patient with idiopathic pulmonary hypertension before and after the pulmonary vasodilator therapy with epoprostenol for three months. Before the pulmonary vasodilator therapy, the RV FDG accumulation was highly increased (**C**). After the therapy, the RV FDG accumulation was markedly decreased (**D**). Reprinted with permission from [55].