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Vascular Metabolic Mechanisms of Pulmonary Hypertension*

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Summary: Pulmonary hypertension (PH) is a severe and progressive disease characterized by increased pulmonary vascular resistance leading to right heart failure and death. In PH, the cellular metabolisms including those of the three major nutrients (carbohydrate, lipid and protein) are aberrant in pulmonary vascular cells. Glucose uptake, glycolysis, insulin resistance, sphingolipid S1P, PGE_2, TXA_2, leukotrienes and glutaminolysis are upregulated, and phospholipid-prostacyclin and L-arginine-nitric oxide pathway are compromised in lung vascular cells. Fatty acid metabolism is disordered in lung endothelial cells and smooth muscle cells. These molecular mechanisms are integrated to promote PH-specific abnormal vascular cell proliferation and vascular remodeling. This review summarizes the recent advances in the metabolic reprogramming of glucose, fatty acid, and amino acid metabolism in pulmonary vascular remodeling in PH and the mechanisms for how these alterations affect vascular cell fate and impact the course of PH.
Key words: pulmonary hypertension; metabolism; vascular remodeling; proliferation

Pulmonary hypertension (PH) is a type of heterogeneous high mortality disease with a mean pulmonary artery pressure (mPAP) >20 mmHg at rest, a pulmonary artery wedge pressure ≤15 mmHg, and a pulmonary vascular resistance (PVR) of ≥3 Wood units[1, 2]. Based on pathophysiology, clinical manifestations and therapeutic approaches, PH is classified into 5 groups[3]. Group 1 is pulmonary arterial hypertension (PAH) that is either idiopathic or associated to heritable gene mutations of bone morphogenic protein receptor type 2 (BMPR2)[4], activin-like receptor kinase-1 (ALK1)[5], potassium channel subfamily K member 3 (encoded by KCNK3 gene)[6], or drug/toxin causes (aminorex and fenfluramine)[7]. Group 2 is PH due to left heart disease, group 3 due to lung disease and/or hypoxia, group 4 due to chronic thromboembolism, and group 5 due to unclear multifactorial etiologies including hematologic disorders (e.g., myeloproliferative disorders), systemic disorders (e.g., sarcoidosis, pulmonary Langerhans cell histiocytosis, lymphangioleiomyomatosis, neurofibromatosis, vasculitis), metabolic disorders (e.g., glycogen storage disease, Gaucher disease, thyroid disorders), or miscellaneous conditions (e.g., tumor obstruction, mediastinal fibrosis, chronic renal failure on dialysis).

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The fundamental causes of different types of PH are varied. However, all patients with PH have pathological features of excessive pulmonary vasoconstriction and abnormal vascular remodeling[8]. Endothelial dysfunctions such as endothelial cell hyperproliferation, enhanced collagen deposition and plexiform lesions narrow the vascular lumen[9]. Pulmonary artery smooth muscle cells (PASMCs) and fibroblast hyperproliferation and apoptosis resistance play critical roles in the progress of vascular remodeling[8]. The thickening of all vessel layers including intima, media and adventitia leads to progressive narrowing and occlusion of the distal pulmonary arteries, increased pulmonary vascular resistance, right ventricular failure or even death[10, 11]. For decades, tremendous of theories have been proposed for the mechanism of PH[12, 13]. However, the molecular mechanisms underlying vascular dysfunction remain unclear. It is particularly important to clarify the common pathological processes of various PH. Pulmonary vasculature undergoes cellular hyperproliferation, apoptosis resistance, inflammation, and relevant metabolic reprogramming in PH[14–17]. The cellular metabolisms including those of the three major nutrients (carbohydrate, lipid and protein) are aberrant in pulmonary vascular cells[18–20]. These metabolic molecular mechanisms are integrated to promote PH-specific abnormal vascular cell proliferation and tissue remodeling. In this review, we will summarize the recent progress in the metabolic

reprogramming of glucose, fatty acid, and amino acid metabolisms in PH and the mechanisms for how these alterations affect vascular cell fate and impact the course of PH.

1 GLUCOSE METABOLISM

Like other organs in human body, the primary source of energy for lung cells is glucose[21]. As the first pathway of glucose catabolism in all cells, glycolysis is a sequence of 10-step reaction which converts glucose into two pyruvate molecules. In this catabolism process, the released free energy is used to form two ATP molecules and two reduced nicotinamide adenine dinucleotide (NADH) molecules. In order to ensure that glycolysis continues, NADH must be re-oxidized to NAD^+. In aerobic environment, NAD^+ will be regenerated in the electron transport chain (ETC) which occurs in mitochondria. In anaerobic environment or cancer cells, pyruvate is converted to lactate by lactate dehydrogenase (LDH), meanwhile NADH is oxidized to NAD^+[22].

In aerobic condition, pyruvate is delivered to mitochondrial matrix by mitochondrial pyruvate carrier (MPC), then decarboxylated by the pyruvate dehydrogenase complex (PDH) to generate acetyl-CoA and finally broken down by the tricarboxylic acid (TCA) cycle. The NADH and flavin adenine dinucleotide (FADH2) generated by the TCA cycle are fed into the oxidative phosphorylation in the ETC. In this case, each glucose molecule produces up to 38 ATPs by aerobic respiration. However, instead of more efficient aerobic respiration, a "Warburg-like effect" has been found in PH patients[23, 24]. Furthermore, dynamic positron emission tomography (PET) imaging scans of ^{18}F-labeled deoxyglucose (^{18}FDG) has demonstrated the increased glucose uptake and metabolism in both PH patients and monocrotaline-induced PH rat model[17, 25, 26].

As a key transcription factor, hypoxia-inducible factor-1α (HIF-1α) plays a crucial role in the pathogenesis of PH. Once activated by triggers of PH, such as hypoxia and metabolic stress, HIF-1α upregulates the transcription of pyruvate dehydrogenase kinase (PDK)[27, 28]. The activated PDK leads to the inhibition of PDH and the suppression of aerobic respiration. LDH in the cytoplasm catalyzes the production of lactate from pyruvate. In the condition of transitory hypoxia, this pathway helps the cells to acquire basic energy and maintain survival. However, the long-term stress environment leads the pulmonary vascular cells to hyperproliferation and vascular remodeling[29]. For more than a decade, PDK and PDH are promising targets for PH treatment. Dichloroacetate (DCA) is a well investigated small molecule inhibitor of PDK that activates PDH and promotes mitochondrial oxidative phosphorylation. Several experiments on animal models of PH have demonstrated that the administration of DCA obviously prevented and reversed pulmonary hypertension[30–33]. McMurtry *et al* reported that DCA reverses pulmonary arterial remodeling via activating mitochondrial-dependent apoptotic pathway and upregulating Kv1.5 in the media layer[30]. Recently, a 4-month, open-label clinical study in patients with idiopathic PAH (IPAH) has been conducted[34]. With approved therapies, DCA administration reduced mean pulmonary artery pressure and pulmonary vascular resistance and improved functional capacity. This work suggests that the metabolic theory of PH is feasible in humans. In addition to PDK and PDH, multiple factors regulate glucose metabolism. Glucose uptake and glycolysis pathways play important roles in angiogenesis. Glucose transporter (GLUT), which mediates glucose uptake, and hexokinase-1 (HK1), which mediates the first step of glycolysis, are both upregulated in pulmonary vascular cells of IPAH patients and PH animal models[35]. DCA and imatinib treatment decreases the GLUT1 protein level in the lungs of PH animal models[26, 35].

Glucose metabolic shift is caused not simply by glucose metabolic flux being passively diverted to glycolysis due to damaged mitochondrial metabolism. It occurs proactively due to increased activities of rate-limiting enzymes in the glycolytic pathway in PH[36, 37]. The 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3 (PFKFB3) is an enzyme that regulates fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F1,6P2), one of the rate-limiting checkpoints in glycolytic flux. Recently, we found that PFKFB3 is upregulated in lungs of patients with IPAH and PH rodents[36, 37]. The PFKFB3 inhibition by genetic disruption and chemical inhibitor attenuated the development of PH in animal models. In PASMCs, PFKFB3 elevated glycolysis and lactate, resulting in hyperproliferation by ERK1/2-dependent activation of calpain-2. Moreover, in pulmonary arterial endothelial cells (PAECs), PFKFB3 indirectly upregulated HIF-2α, leading to PAEC release of growth factors and inflammatory cytokines and the development of PH[36, 37]. Pyruvate kinase muscle isozyme M1/2 (PKM1/2) is a rate-limiting enzyme in glycolysis pathway that catalyzes phosphoenolpyruvate (PEP) to pyruvate. PKM2 is a prolyl hydroxylase 3 (PDH3) stimulating coactivator, which interacts with HIF-1α. HIF-1α in turn activates transcription of genes encoding PHD3, PKM2, GLUT1, LDHA, PDK1, and other metabolic enzymes that mediate the Warburg effect[38]. In PH, it has been shown that the splicing factor polypyrimidine-tract-binding protein (PTBP1) and the ratio of PKM1/PKM2 are upregulated, which mediate the metabolic and proliferative abnormalities caused by the downregulation of miR-124 in pulmonary

artery endothelial cells and fibroblasts[14, 24, 29].

Another metabolic pathway of glucose is hexosamine biosynthesis pathway (HBP), which coordinates with amino acid, fatty acid and nucleotide metabolism. Uridine-diphosphate-β-D-N-acetylglucosamine (UDP-GlcNAc), the production of HBP, serves as a substrate of hyaluronan (HA) and O-linked β-N-acetylglucosamine (O-GlcNAc) modification of proteins[40]. Barnes *et al* reported that the HBP flux is increased in IPAH patients. Further research found that enhanced HBP flux upregulates *O*-linked β-*N*-acetylglucosamine transferase (OGT), which in turn activates host cell factor-1 (HCF-1), resulting in excessive proliferation of PASMCs[41–43].

2 INSULIN RESISTANCE (IR) AND PH

IR is a key feature of type 2 diabetes mellitus (T2DM). IR is mainly manifested as inhibited hepatic glucose production and impaired glucose clearance of peripheral tissues. Hyperinsulinemia is an important marker of IR[44]. Recent studies and epidemiological investigations have found that IR is closely related to PH and potentially an important risk factor for the development of PH[45–47]. Apolipoprotein E knockout mice fed on a high-fat diet (HFD) developed IR and PH. In addition, IR and PH were reversed when treated with rosiglitazone, a highly effective insulin sensitizer[48, 49]. West *et al* found that IR and blood glucose homeostasis dysfunction are present as early features in Bmpr2 mutant mice. Moreover, insulin-mediated endothelial glucose uptake is impaired by reduced glucose transporter translocation[50, 51]. Compared to lean littermates, Zucker diabetic fatty (ZDF) rats which have a missense mutation in the gene coding the leptin receptor show signs of PH, such as elevated pulmonary arterial pressure (PAP), right ventricle hypertrophy, and remodeling of the vessel structure[52]. Epidemiological studies have also shown that patients with IPAH have a higher rate of impaired glucose tolerance (57% *vs*. 14%) and that IR in PH is characterized by increased hemoglobin A1c (HbA1c) and alterations in lipid and lipoprotein homeostasis axes[53, 54]. Diabetic PH patients are more likely to have pulmonary vein disease and worse long-term survival than non-diabetic PH patients[55].

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that regulates glucose and lipid metabolism. PPARγ is involved in the pathogenesis of a variety of diseases, including obesity, diabetes, atherosclerosis, and cancer[56, 57]. In the lung tissue of patients with PAH, the expression of PPARγ is reduced[58, 59]. There is evidence that PPARγ is a protective regulator of PH[59, 60]. PPARγ agonist thiazolidinediones (TZDs) are recognized insulin sensitizers for the treatment of T2DM and metabolic syndrome. TZDs such as rosiglitazone and GW9662 attenuate phenotype in several animal models of PH[48, 61, 62]. However, none of these animal models show symptoms of IR, meaning that the effects of TZDs on PH are independent of IR. Calvier *et al* found that PPARγ is a key regulator between BMP2 and TGFβ1 in PASMCs[63]. PPARγ activation rescues BMPR2 dysfunction, inhibits transforming growth factor-β1 (TGF-β1) pathway (TGFβ1-Stat3-FoxO1 and TGFβ1-Smad3/4) in PASMCs via interactions with SMAD3 and STAT3. Furthermore, TGF-β1 pathway suppresses PPARγ by miR-130/301, a key miRNA family that regulates metabolism and cell proliferation, in pulmonary vascular cells. Conversely, the activation of BMP2/BMPR2-PPARγ axis inhibits TGFβ1-induced PASMC glycolysis and proliferation by upregulating miR-148a (a suppressor of proliferation) and miR-331-5p (a suppressor of PFKP–a key enzyme of glycolysis)[60, 63–65]. Others found that PPARγ inhibits PDGF-induced PASMC proliferation via HDAC1/miR-124/CDK4 axis[66]. Furthermore, deletion of PPARγ reduces PPARγ coactivator 1α (PGC-1α) and causes mitochondrial dysfunction and PASMC proliferation[67]. Taken together, the role of PPARγ in metabolic regulation of PH is beneficial, and FDA-approved drugs, rosiglitazone and pioglitazone, hold promise for the treatment and prevention of PAH and right ventricular failure[62, 68].

3 FATTY ACID B-OXIDATION (FAO) AND PH

β-oxidation is the major catabolic process of fatty acid molecules that occurs in mitochondrial matrix. Through this process, fatty acid molecules are broken down into acetyl-CoA which enters the TCA cycle producing NADH and FADH2 which enter the ETC. For eukaryotes, β-oxidation is an excellent pathway to produce ATP, whereas β-oxidation consumes 12% more oxygen than glycolysis to yield the same amount of ATP. Emerging evidence suggests that fatty acid metabolism is abnormal in PH patients and rodent models. Patients with PAH exhibit elevated circulating free fatty acids (FFAs) and long-chain acylcarnitines as well as FA accumulation in the myocardium[69–72].

Gene array showed that several enzymes of FAO such as fatty acetyl CoA L1 (ACSL1), acyl-CoA dehydrogenase (ACADM) and acetyl-CoA acetyl transferase 1 (ACAT1) are increased in the lungs of PAH patients[73]. The Randle cycle is described as a competitive relationship between glucose oxidation and FAO[74]. Citrate generated by FAO inhibits phosphofructokinase (PFK) and HK, which in turn inhibits glycolysis. Moreover, acetyl-CoA generated from FAO inhibits PDH, thereby inhibits pyruvate entrance into the TCA cycle. Based on the Randle cycle, activation of glucose oxidation by inhibiting FAO might

be beneficial in PH treatment. Inhibition of malonyl-CoA decarboxylase (MCD) can inhibit the entrance of fatty acids into the mitochondria, in turn decreasing FAO, thus activating PDH and glucose oxidation. Sutendra *et al* showed that MCD deficient mice are protected from hypoxia-induced PH. Treatment with trimetazidine that mimics the lack of MCD reverses the PH induced by hypoxia or monocrotaline in mice and rats[75, 76]. In addition, carnitine palmitoyltransferase I (CPT1), a rate-limiting enzyme in FAO, promotes PASMC proliferation by downregulating the AMPK-p53-p21 pathway[77]. At present, the strategy of suppressing FAO has not been verified in clinical trials for PH.

4 ADIPOKINE (LEPTIN AND ADIPONECTIN) AND PH

Leptin is a hormone predominantly secreted by adipocytes, which plays an important role in suppressing appetite, regulating energy consumption[78]. Recently, increasing evidence demonstrates that leptin is involved in vascular smooth muscle cell proliferation, endothelial dysfunction and platelet aggregation[79–81]. It is reported that leptin is upregulated in the pulmonary vasculature of IPAH patients and PH animal models[82]. Notably, *ob/ob* mice (a leptin knockout model of T2DM) are protected from hypoxia-induced PH. However, the role of leptin in PH is still inconclusive. Both *ob/ob* mice and ZDF rats show PH features, such as elevated PAP, pulmonary arterial remodeling and right ventricular hypertrophy[52, 83]. Regulation of leptin activity for the treatment of PH still needs further investigation.

Adiponectin is involved in glucose and lipid metabolism that is synthesized by the adipocytes as well. Adiponectin knockout mice showed an age-dependent increase in pulmonary arterial pressures[84]. Emerging evidence demonstrates that adiponectin inhibits the dysfunction and proliferation of vascular endothelial cells and smooth muscle cells by regulating the synthesis of nitric oxide (NO) and mediating AMPK, mTOR and NF-κB signaling[49, 85, 86]. In addition, as a target gene of PPARγ, adiponectin level is upregulated by PPARγ agonists in PH models[60, 62]. Adenovirus overexpression of adiponectin can also suppress hypoxic-induced pulmonary vascular remodeling[87].

5 SPHINGOLIPID METABOLISM

Sphingolipids are not only important components of plasma membrane but also bioactive molecules that play an important role in cell recognition and signal transduction. Sphingosine 1-phosphate (S1P) is a bioactive lipid mediator. Sphingosine kinases 1 and 2 (SphK1 and SphK2) phosphorylate sphingosine to form S1P. S1P acts as a second messenger and directly interacts with intracellular proteins. It is also transported to the outside of cells and binds to a family of S1P-specific G-protein-coupled receptors ($S1P_{1-5}$) and activates a series of signaling pathways involved in cell proliferation, apoptosis, inflammation and migration[88, 89]. Chen *et al* found that Sphk1 and S1P are upregulated in patients and rodents with PH[90]. Both SphK1 deficiency and pharmacologic inhibition (JTE013) protect against hypoxia-induced PH in rodents. In addition, S1P ligates S1PR2, leading to PASMC proliferation via activation of both ERK1/2 and STAT3 signaling[90]. Another study found that administration of PF-543 (a SphK1 inhibitor) in hypoxia-induced PH mice had no effect on vascular remodeling but reduced right ventricular hypertrophy[91]. However, in endothelial cells, inhibition of Sphk1 induces endothelial dysfunction, suggesting that Sphk1 plays a protective role in the endothelium[92]. Inhibition of Nogo-B (a negative regulator of S1P biosynthesis) upregulates the S1P-$S1P_1$-endothelial nitric oxide synthase (eNOS) signaling axis, leading to NO production and vasodilation[93]. The opposite effect of Sphk1 and S1P in smooth muscle cells and endothelial cells makes it difficult to become an excellent therapeutic target for PH. A viable strategy is to develop inhibitors that inhibit SphK1 in vascular smooth muscle cells while having no effect on endothelial cells[94].

6 PHOSPHOLIPID-ARACHIDONIC ACID METABOLISM

Phospholipids are a class of lipids that are a major component of all cell membranes. Arachidonic acid (AA) is freed from a phospholipid molecule by the enzyme phospholipase A_2. AA is converted to prostaglandin H_2 (PGH_2) by cyclooxygenase (ubiquitous COX-1 or inducible COX-2). PGH_2 is then catalyzed to prostaglandin E_2 (PGE_2), thromboxane A_2 (TXA_2), prostacyclin (PGI_2) and others. AA is also metabolized by 5-lipoxygenase into 5-hydroperoxyeicosatetraenoic acid (5-HPETE), which in turn is converted to various leukotrienes.

COX-2 can be induced in smooth muscle of pulmonary vessels by hypoxia[95]. Knockdown of COX-2 exacerbates hypoxia- and monocrotaline-induced PH and enhances contractility of PASMCs[96, 97]. PGE_2 and TXA_2 evoke contraction of pulmonary arteries through the E prostanoid 3 receptors (EP_3) and thromboxane prostanoid (TP) receptors, respectively[98, 99]. PGE_2 generation and EP_3 expression are increased in PASMCs and pulmonary arteries in response to hypoxia, and in pulmonary arteries from PH animal models. Disruption and blockade of EP_3 attenuate pulmonary hypertension and pulmonary vascular remodeling in PH animal models[100]. Blockade of TP-mediated signaling

significantly suppresses the hypoxia-induced hyper-reactivity of the pulmonary arteries[101]. Prostacyclin binds to IP receptor on vascular smooth muscle cells, and then triggers the activation of adenylate cyclase and increases intracellular cyclic AMP (cAMP), leading to vasodilation[102]. Prostacyclin plays an important role in the pathogenesis and treatment of PH. It is demonstrated that the ratio of thromboxane A_2/prostacyclin is increased in patients with PH since prostacyclin synthase is decreased[103, 104]. Hypoxia decreases prostacyclin production in lung endothelial cells[105]. Furthermore, prostacyclin synthase over-expression ameliorates PH in several rodent models[106–109]. Notably, prostacyclin and its analogs such as treprostinil, iloprost and beraprost are used for treatment of PAH[110, 111]. In addition to vasodilatory effects, prostacyclin and its analogs also have anti-proliferative and pro-apoptotic effects on PASMCs[112, 113]. This suggests that activating prostacyclin pathway can reverse pulmonary vascular remodeling.

Leukotrienes are potent chemoattractants for multiple inflammatory cell types, especially neutrophils and macrophages and modulate inflammation in PAH[114, 115]. PAH lungs have elevated protein levels of both 5-lipoxygenase and 5-lipoxygenase activating protein (FLAP) and increased 5-lipoxygenase mRNA levels[116]. Over-expressing 5-lipoxygenase markedly accelerated the progression of monocrotaline PAH[117]. Leukotriene B_4 induces PAEC apoptosis and the proliferation and hypertrophy of PASMCs[118]. Blockade of leukotrienes reversed PH and prevented PH-related death[115, 119, 120]. Thus upregulation of 5-lipoxygenase and leukotrienes promotes PH.

7 AMINO ACID L-ARGININE METABOLISM

In vascular endothelium, L-arginine is converted into NO, L-citrulline and H_2O via a reaction catalyzed by eNOS. NO as a powerful endothelium-derived vasodilator plays an important role in the low vascular resistance in the pulmonary circulation[121, 122]. The increase in PAP and pulmonary vascular remodeling associated with hypoxic PAH are attributable to reduction of NO release from hypoxic pulmonary endothelium. The studies from cultured pulmonary endothelial cells, animal models, and human subjects have confirmed that hypoxia reduces NO production from lung endothelium[123]. The reduced NO release in PH is contributed to compromised L-arginine cellular uptake[124], decreased eNOS activity[123, 125] and increased NO decomposition[126].

After synthesis in vascular endothelial cells, NO diffuses into adjacent smooth muscle cells to activate soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) signaling[127]. cGMP acts as a secondary messenger and activates cGMP-dependent protein kinase (PKG) to promote vasodilation and inhibit proliferation of vascular smooth muscle cells[128]. Riociguat, an activator of sGC, has been approved by FDA to treat two types of PH: chronic thromboembolic pulmonary hypertension (CTEPH) and IPAH[129, 130]. In addition, phosphodiesterase-5 (PDE5) can degrade cGMP into 5′-GMP, thereby inhibiting vasodilation. Of note, two PDE5 specific inhibitors, sildenafil and tadalafil, are approved for PH therapy[131–134].

As the precursor of NO, L-arginine is mainly produced by internal protein decomposition. L-arginine also can be synthesized from L-citrulline in a sequential action catalyzed by argininosuccinate synthetase (ASS) and argininosuccinate lyase (ASL)[135]. Erez *et al* showed that hypomorphic ASL deficiency leads to reduced NO synthesis in mice. Administration of nitrite can rescue the manifestations of NO deficiency in hypomorphic ASL mice[136]. In addition to forming NO, L-arginine also participates in the urea cycle, producing urea and ornithine by the catalysis of arginase. Arginase regulates NO synthesis by competing with NOS for the substrate L-arginine[137]. It has been found that arginase was upregulated in PAECs of PAH patients[138]. Furthermore, administration of arginase inhibitors prevented the progression of PH in animal models[139, 140]. Cowburn *et al* found that HIF-2α causes upregulation of arginase and dysregulation of NO synthesis[141]. Moreover, as an endogenous inhibitor of the NOS, ADMA blunts NO synthesis by competing with L-arginine for the active site of NOS[142].

8 AMINO ACID GLUTAMINE METABOLISM

In mitochondria, glutaminase (GLS) catalyzes the conversion of glutamine to glutamate and ammonia. Glutamate is then converted to α-ketoglutarate (α-KG), a TCA cycle intermediate, by glutamate dehydrogenase (GDH) or alanine or aspartate aminotransferase (ALT, AST) to produce ATP and biosynthetic materials. Excessive glutaminolysis is observed in hyperproliferative cells such as cancer cells and PH vascular cells[143–145]. PAH patients with BMPR2 mutations exhibit a shift of glutamine metabolism, mainly characterized by increased glutamine uptake in lung vasculature of PAH patients[146]. Bertero *et al* reported that the downstream effectors of Hippo pathway, the transcriptional co-activators Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), upregulate GLS1, leading to glutaminolysis and hyper-proliferation of pulmonary vascular cells. Pharmacologic inhibition of either YAP or GLS1 alters glutaminolysis and pulmonary vascular cell proliferation in monocrotaline-induced rat PH model[147]. Furthermore, Dumas *et al* reported that glutamate accumulation in PH stimulates the glutamate receptor N-methyl-D-aspartate receptors (NMDAR), promoting

PASMCs proliferation induced by type A-selective endothelin (ETA) receptor and platelet-derived growth factor (PDGF) receptor[144]. Both genetic deficiency and pharmacologic inhibition of NMDARs attenuate the development of PH in rodent models[144]. Based on these dramatic findings, intervention of glutamine metabolism provides a promising strategy for PH.

9 CONCLUSION

In PH, the cellular metabolisms including those of the three major nutrients (carbohydrate, lipid and protein) are aberrant in pulmonary vascular cells (table 1). Glucose uptake, glycolysis, IR, sphingolipid S1P, PGE_2, TXA_2, leukotrienes and glutaminolysis are upregulated, and phospholipid-prostacyclin and L-arginine-NO pathway are compromised in lung vascular cells. Fatty acid metabolism is disordered in lung endothelial cells and smooth muscle cells. These molecular mechanisms are integrated to promote PH-specific abnormal vascular cell proliferation and remodeling. Although pharmacotherapy such as endothelin-1 receptor antagonists, PDE5 inhibitors, and prostacyclin analogues have been developed, targeting these aberrant metabolic pathways will open new venue to novel PH therapies.

Table 1 Metabolic abnormalities in PH and their potential targeting agents

Metabolic changes	Targeting agents
Glucose metabolism	
PDK and PDH↑	Dichloroacetate
PKM1/2↑	
GLUT1↑	
PFKFB3↑	3PO, PFK15
Hexosamine biosynthesis↑	
PPARγ↓	Rosiglitazone, pioglitazone and GW9662
Lipid metabolism	
MCD↑	CBM-301106
Abnormal Leptin, Adiponectin	
SphK1/S1P↑	JTE013 and PF-543
PGE_2↑	L-798106
TXA_2↑ and prostacyclin↓	Treprostinil, Iloprost and Beraprost (prostacyclin analogs)
	Selexipag (prostacyclin receptor agonist)
Leukotrienes↑	Bestatin, JNJ-26993135, LY293111
Amino acid metabolism	
L-Arginine-nitric oxide↓	Sildenafil and tadalafil (PDE inhibitors)
	S-boronoethyl-L-cysteine and nor-NOHA (arginase inhibitors)
Glutaminolysis↑	BPTES, CB-839
YAP/TAZ↑	Verteporfin
NMDAR↑	MK-801

PDK, pyruvate dehydrogenase kinase; PDH, pyruvate dehydrogenase complex; PKM1/2, pyruvate kinase muscle isozyme M1/2; GLUT1, glucose transporter 1; PFKFB3, 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase 3; PPARγ, peroxisome proliferator-activated receptor γ; MCD, malonyl-CoA decarboxylase; SphK1, sphingosine kinases 1; S1P, sphingosine 1-phosphate; PGE_2, prostaglandin E_2; TXA_2, thromboxane A_2; YAP/TAZ, transcriptional co-activators Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif; NMDAR, N-methyl-D-aspartate receptor

Conflict of Interest Statement

The authors have no conflict of interest.

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