A Proposed Mitochondrial–Metabolic Mechanism for Initiation and Maintenance of Pulmonary Arterial Hypertension in Fawn-Hooded Rats: The Warburg Model of Pulmonary Arterial Hypertension

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Abstract Pulmonary arterial hypertension (PAH) is a disease of the pulmonary vasculature that is characterized by vascular obstruction and progressive right ventricular failure. One hallmark of clinical PAH is its very poor survival, with PAH mortality rates approximating those of many malignancies. The discovery that the fawn-hooded rat strain (FHR) spontaneously develops PAH has allowed for major insights into the pathophysiology of PAH. These findings have revealed that cancer and PAH not only share a similarly poor prognosis but also demonstrate similar resistance to apoptosis and activation of cell proliferation as a major pathophysiologic mechanism. One of the causes for the resistance to apoptosis and increased proliferation of pulmonary vascular smooth muscle cells in PAH is a cancer-like metabolic shift towards a glycolytic metabolism (Warburg effect) and down-regulation of mitochondrial glucose oxidation. This book chapter will review the role of such a metabolic shift in the pathophysiology of PAH and also highlight emerging anti-proliferative PAH therapies that correct the metabolic dysregulation in PAH.

Keywords pulmonary arterial hypertension (PAH) • vascular smooth muscle cell proliferation Warburg effect • cancer • epigenetic silencing • reactive oxygen species • mitochondrial electron transport chain • fawn-hooded rats (FHR) • pyruvate dehydrogenase kinase • glycolysis • hypoxia-inducible factor-1α

1 Introduction

Pulmonary arterial hypertension (PAH) is a disease of the pulmonary vasculature, which occurs in a rare idiopathic form (sporadic PAH-90%, familial PAH-10%) and, more commonly, as a syndrome associated with connective tissue diseases,

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congenital heart disease, anorexigen use, portopulmonary disease, or HIV. The reported prevalence of idiopathic PAH (iPAH) (1/1,000,000) is likely an underestimation, due to lack of data on sickle cell and schistosomiasis-associated PAH syndromes in Africa and Asia, and to insensitivity of the history and physical examination, as suggested by the high prevalence of moderate pulmonary hypertension in surveillance studies of high-risk cohorts with connective tissue diseases.[1] Despite important advances, such as the discovery of mutations in bone morphogenetic protein receptors (BMPR2) as a cause of familial PAH and the advent of effective oral therapies, such as phosphodiesterase 5 inhibitors and endothelin antagonists, mortality remains high (15% at 1 year).[2] Lack of a cure reflects ignorance of the cause of PAH and the related absence of optimally targeted therapies.

2 An Emerging Paradigm: The "Oncologic" View of Pulmonary Artery Hypertension

Recently several groups have concluded that PAH may be viewed, in part, as a disease of excess proliferation and impaired apoptosis of pulmonary artery smooth muscle cells (PASMC),[3–6] similar in some regards to neoplasia.[7,8] Similarities between PASMC in PAH and cancer cells include increased rates of cell proliferation, depressed rates of apoptosis, pathological activation of hypoxia-inducible factor 1α (HIF-1α) as well as metabolic shift towards glycolysis which is characterized by activation of pyruvate dehydrogenase kinase (PDK)[8] and downregulation of voltage-gated potassium channels (K_v)[9] (reviewed in Ref.[10]). Recently, we discovered that strategies which regress experimental PAH[8] such as PDK inhibition and K_v1.5 gene therapy also regress human cancers,[7] thus further highlighting that cancer and PAH may share some underlying pathophysiological pathways. While excessive vasoconstriction,[11–14] inflammation[15] and thrombosis[16] contribute to the pathogenesis of PAH, this new "oncologic view" of PAH, originally proposed by Tuder and Voelkel,[17] may constitute a paradigm-shift from the twentieth-century view of PAH as a primarily vasospastic disease.

3 Overview of Existing Mechanisms of PAH

Although the "oncologic" view of PAH emphasizes the critical role of PASMC hyperproliferation, it is important to recognize that PAH is a panvasculopathy. Abnormalities in each layer of the blood vessel contribute to this syndrome of obstructed, constricted small pulmonary arteries (PA), which ultimately results in right ventricular hypertrophy (RVH). Indicators of endothelial involvement in PAH include elevated plasma serotonin[18] and a decreased ratio of vasodilators/constrictors.[19–21] It is also hypothesized that widespread endothelial apoptosis in early PAH culminates in selection of apoptosis-resistant endothelial precursor cells that proliferate and eventually form plexiform lesions.[22] In the media of the

pulmonary artery, three groups, Rabinovitch's,[6,23] Yuan's[5,24,25] and ours[26–28] have independently shown that PASMC apoptosis is suppressed and proliferation is enhanced in experimental PAH, which is consistent with the findings in human PAH.[29] Many factors drive PASMC proliferation, including mutation[29] or downregulation[30] of BMPR-2, de novo expression of the anti-apoptotic protein survivin,[22,28] increased expression/activity of the serotonin transporter (SERT)[31,32] and increased expression/activity of platelet-derived growth factor (PDGF) receptor.[33] Another proliferative, anti-apoptotic PASMC abnormality is the selective decrease in expression of $K_v1.5$, a voltage-gated, O_2-sensitive potassium channel. The downregulation of $K_v1.5$, which is also the channel that is inhibited by hypoxia and initiates hypoxic pulmonary vasoconstriction,[34,35] is a hall-mark of PAH and occurs in human PAH,[36] experimental PAH models (induced by chronic hypoxia[26,37] or monocrotaline[28)] or genetic predisposition to PAH such as the fawn hooded rat[8] and transgenic mice with PAH due to overexpression of SERT[32] or a BMPR-2 dominant negative mutation.[38] In PAH, loss of $K_v1.5$, depolarizes the membrane and elevates cytosolic levels of K^+ and Ca^{2+}. The resulting calcium overload, which is later reinforced by activation of TRP channels,[39] leads to Ca^{2+}-calcineurin-dependent activation of the proliferative transcription factor, NFAT,[40] thus pointing to a causal role of $K_v1.5$. In the adventitia, metalloprotease activation causes architectural disruption, permitting cell migration and generating mitogenic peptides (tenascin).[23] Finally, infiltration of the lung with inflammatory cells, endothelial-precursor cells, and mesenchymal and bone-marrow-derived stem cells occurs in PAH,[41] and these cells may contribute to the syndrome.

With the discovery of BMPR-mutations in familial PAH,[42,43] the cause of PAH appeared to have been elucidated. These loss-of-function mutations favor PASMC proliferation and consistent with this model, a transgenic mouse with SMC-specific over-expression of a human dominant-negative BMPR2 transgene develops PAH.[44] Interestingly, PAH in BMPR-2 dominant-negative mice is initially not associated with vascular remodeling,[38] but manifests $K_v1.5$ deficiency and can be reversed with an L-type calcium channel blocker.[38] This suggests that disordered BMP signaling, leading to reduced $K_v1.5$ transcription, may be a link between an early vasospastic stage of familial PAH and the later fixed anatomic pathology. Eventually, impaired apoptosis and enhanced PASMC proliferation transforms PAH to a more fixed disease.[27] Indeed only 30–40% of PAH patients have significant vasodilator responses (>20% fall in PVR and PAP) to inhaled nitric oxide,[45,46] which points to vascular remodeling being a key component of the pathology in the majority of PAH patients. However, BMPR2 mutation does not appear to be a common cause of sporadic PAH. BMPR-2 mutations occur in only 10–20% of sporadic PAH patients and even in familial PAH, penetrance is low (~20%).[47] While modifier genes, such as CYP1B1, SERT and TGF-β may explain variable penetrance, aberrant BMPR2 function alone is neither a necessary nor sufficient precondition for most cases of PAH.[47] This literature highlights the multiplicity of putative "causes" for PAH and, in so doing, highlights the lack of a fundamental, initiating cause for nonfamilial PAH. While this partially reflects the fact that PAH is a syndrome, rather than a homogenous disease, it raises the question, "Does an additional unifying cause for PAH exist?" We believe the answer is yes and hypothesize that upstream mitochondrial abnor-

malities lead to metabolic dysregulation which in turn creates a downstream proliferative, anti-apoptotic phenotype.[8] This unifying model integrates the observed metabolic abnormalities in PAH with the critical increase in PASMC proliferation and vascular remodeling and is based on both experimental models of PAH in fawn hooded rats (FHR) and data from humans with PAH.

4 The Fawn-Hooded Rat as a Model for Idiopathic Pulmonary Artery Hypertension

The fawn hooded rats (FHR) are a mutant strain, named for their brown mantle of fur. FHR spontaneously develop PAH.[48] In studying PAH in FHR it is useful to compare FHR to consomic control rats. Consomic rats (FHR-BN1 control rats) were created by introgression of the hypoxia-resistant, Brown Norway rat's chromosome 1 into an isogenic FHR background using marker-assisted selection.[49] Other than chromosome 1 substitution, the consomics are identical to FHR. Importantly, the control FHR-BN1 strain does not develop PAH.[8]

The original FHR were an outbred strain created from "German brown," Lashley and Wistar albino, and Long Evans rats. Three major FHR strains are known: PAH prone FHH/EurMcwiCrl (which we use), a systemically hypertensive FHH strain (prone to glomerulonephritis) and a strain prone to depression/substance addiction. PAH in FHR is heritable and occurs in males and females.[50] In addition, FHR are hypoxia-sensitive, developing PAH and alveolar simplification when exposed to mild hypoxia, at levels that do not affect normal rodents.[50] FHR show exaggerated vasoconstrictor responses when raised at high altitudes.[12] The natural history of FHR likely varies based on concomitant hypoxic exposure. In Denver (elevation 5,200 ft) PAH develops within 1 month of birth.[50] In contrast, in Edmonton, Alberta, at roughly half the altitude, PAH develops at a similar prevalence, but later in life (between 20 and 40 weeks). PAH is ultimately lethal in FHR by ~60 weeks.[8] FHR PASMC share with human PAH PASMC, an exaggerated predilection to proliferate[8,29] and excessive rho kinase activity.[12] Additional similarities with human PAH include enhanced vasoconstriction to serotonin and a platelet storage-pool deficiency.[51] These characteristics of the FHR make it an excellent model to study the pathophysiology of PAH as well as develop novel treatment strategies.

5 A Metabolic Axis of Pulmonary Artery Hypertension

We recently discovered that FHR and humans with iPAH share an unexplained PASMC mitochondrial–metabolic phenotype that underlies their proliferation/apoptosis imbalance. PAH PASMC are characterized by: a fragmented, hyperpolarized

mitochondrial reticulum, decreased superoxide dismutase-2 (SOD2) expression/activity, a metabolic shift away from oxidative metabolism and normoxic activation of the transcription factor HIF-1α (hypoxia inducible factor-1) and the enzyme pyruvate dehydrogenase kinase (PDK). This dysregulation of the metabolic *SOD2-H_2O_2-HIF1-PDK* pathway increases proliferation and suppresses apoptosis in pulmonary artery smooth muscle cells. Since PAH patients demonstrate similar SOD2 downregulation and HIF-1α activation,[52,53] characterization of this metabolic-mitochondrial pathway has significant translational relevance that could impact the development of novel PAH therapies. This chapter reviews recent progress in identifying the mechanisms underlying these PASMC mitochondrial-metabolic abnormalities and in testing their potential as therapeutic targets. The discussion focuses on three key, related mitochondrial-metabolic abnormalities that not only contribute to PAH progression, but may also form a key underlying cause of idiopathic PAH: (1) Epigenetic silencing of SOD2, (2) Activation of the transcription factor HIF-1α and (3) Activation of PDK and glycolytic metabolism. These findings of metabolic dysregulation have paved the way for novel therapeutic strategies which restore SOD2 activity or inhibit HIF-1α and PDK, thus leading to a reduction in SMC proliferation and a regression of PAH.

5.1 Epigenetic Silencing of SOD2 and the Role of H_2O_2 in PAH

SOD2 is located in the mitochondria and is a major source of endogenous H_2O_2. At physiologic levels, H_2O_2 is a vasodilatory and antiproliferative redox-signaling molecule.[54–57] H_2O_2 is produced in mitochondria where SOD2 detoxifies the low basal amounts of superoxide generated by unpaired electron flux during normal activity of the electron transport chain (ETC). Several observations lead us to investigate the importance of SOD2, a mitochondrial protein that is encoded by a gene on rat chromosome 1. First, consomic control rats (FH-BN1), which are identical to FHR save for introgression of a normal chromosome 1 have normal SOD2 levels and do not develop PAH.[8] Second, serial DNA microarray analysis of resistance pulmonary arteries indicates that SOD2 mRNA is downregulated threefold in FHR, prior to onset of pulmonary hypertension. This suggests SOD2 as a candidate PAH gene. Normally, SOD2 expression is induced or repressed to match mitochondrial superoxide production (more superoxide = more SOD2). Consistent with this, oxidant stresses, such as ionizing radiation, induce SOD2 expression in normal animals/cells.[58] This avoids damage to the ETC and mitochondrial DNA. In FHR, decreased SOD2 expression/activity reduces H_2O_2 production.[8] Preliminary data show that restoring mitochondrial H_2O_2, by SOD2 replacement therapy, inhibits PASMC proliferation and enhances apoptosis (unpublished data-not shown). In this context it is critical to realize that H_2O_2 plays an important role in regulating cellular processes such as proliferation and that under-production of this signaling molecule can be harmful. Some groups have also found that in PAH induced by chronic hypoxia the levels of ROS can be increased,[59–61] which underscores the

importance of carefully distinguishing between the effects of specific types of ROS, methods to assess ROS, cellular sources of ROS as well as the dual role of ROS as cause and consequence of PAH during distinct phases of disease progression.

Our unpublished data also suggest that the SOD2 gene and promoter are normal in FHR. This, coupled with the heritable nature of FHR PAH, raises the possibility of epigenetic mechanisms for the SOD2 downregulation. Interestingly, epigenetic silencing of SOD2 is common in many cancers. The SOD2 gene is a putative tumor-suppressor gene (decreased expression is associated with proliferation of cancer cells).[62,63] Epigenetic silencing of SOD2, caused by hypermethylation of CpG dinucleotides within the SOD2 promoter, decreases SOD2 levels in multiple myeloma and pancreatic carcinoma.[64–66] SOD2 downregulation in breast cancer occurs by this mechanism plus a second epigenetic mechanism-histone hypoacetylation. Changes in chromatin acetylation create a repressive chromatin structure that impairs binding of SOD2 transcriptions factor, such as SP-1 and AP-1.[67] HDAC inhibitors, such as trichostatin A and sodium butyrate, restore SOD2 expression in cancer cells.[67] Thus, two epigenetic mechanisms of regulating SOD2 transcription collaborate to control SOD2 expression in cancer.[68,69] In breast cancer and other tumors restoration of SOD2 increases H_2O_2 and limits tumor growth.[65–67] In light of the similarities between cancer and PAH in regards to hyperproliferation of cells, it is likely that similar mechanisms of epigenetic SOD2 silencing may contribute to PASMC hyperproliferation, and our unpublished data suggest that epigenetic downregulation is indeed the cause of reduced SOD2 activity in PAH. This novel finding further points to pharmacological modulation of the epigenetic SOD2 suppression as a potential means for reducing PASMC proliferation and vascular remodeling in PAH.

5.2 HIF-1α Activation and the "Pseudohypoxic" State in PAH

Cancer cells are known to primarily use glycolysis as a source of energy and downregulate mitochondrial activity even in the presence of normal oxygen levels and thrive in this "pseudohypoxic" state. This seminal observation is named the "Warburg effect" because it was made in 1923 by the German scientist and Nobel Prize laureate Otto von Warburg, who also believed that this metabolic switch contributed to the progression of the malignant disease. Even though this observation was made nearly a century ago, the underlying mechanisms of this "pseudohypoxic" state have only recently been elucidated and point to abnormal mitochondrial oxygen-sensing and abnormal activation of the oxygen-sensitive transcription factor HIF-1 as mediators of the "pseudohypoxic" state. Interestingly, this "pseudohypoxic" activation is not only found in cancer cells but also in PAH, and appears to contribute to the pathological cell hyperproliferation in both diseases.

The mitochondrial ETC is the cell's major source of H_2O_2, most of which comes from superoxide anions, produced at complexes I and III, which are converted to H_2O_2 by SOD2.[70] H_2O_2, by virtue of its less toxic nature and moderate diffusion radius, serves as a physiological signaling molecule[71] communicating the "PO_2"

(sensed in the mitochondria) to the plasma membrane (K_v channels[35]) and to transcription factors, notably HIF-1α.[8] We (and others) have demonstrated a PO_2-sensitive ROS production in rodent PA (more oxygen = more ROS).[8,37,70,72–75] This ability to rapidly alter production of the relatively stable ROS H_2O_2 in direct proportion to PO_2 over a physiological PO_2 range (30–100 mmHg)[70,73] is unique to the PASMC mitochondria from small PAs (for example, ROS increase with hypoxia in renal artery SMC).[55] The fact that this superoxide production (and the associated H_2O_2 production by SOD2) is linked (through electron flux) to respiration[76] allows the mitochondria to serve as a cellular "O_2-sensor." Thus, "normoxia" is really a reflection of mitochondrial ROS production and does not always correlate with PO_2. This disconnect is evident for example in cancer or PAH, where low mitochondrial activity reduces superoxide levels and downstream hydrogen peroxide levels are further reduced by low SOD2 expression/activity, despite normal PO_2.

One of the targets of this "pseudohypoxic" state and reduction in H_2O_2 levels is activation of the transcription factor HIF-1α. HIF-1α is a heterodimeric transcription factor, consisting of HIF-1α and HIF-1β subunits.[77] Activation of HIF-1α switches metabolism to glycolysis by activating a panel of glycolytic genes while simultaneously suppressing the activity of the ETC by transactivating the PDK gene, thereby inhibiting the mitochondrial Krebs' (TCA) cycle.[78] Furthermore, HIF-1α activation decreases $K_v1.5$ expression, creating depolarized, calcium-overloaded FHR PASMCs with a proliferative, anti-apoptotic, phenotype[8] with increased activity of the proliferative transcription factor, nuclear factor activating T cells (NFAT).[40] Moreover, HIF-1α haploinsufficient mice are relatively resistant to chronic hypoxic pulmonary hypertension and do not develop downregulation of $K_v1.5$,[79] thus pointing to a causal role of the HIF-1α-$K_v1.5$. pathway in the pathogenesis of PAH. We speculate that the inappropriate normoxic activation of HIF-1α found in FHR and human PAH is triggered by a loss of endogenous H_2O_2. Other groups have also found, that H_2O_2 inactivates HIF-1α,[80,81] as do we.[8]

5.3 PDK Activation in PAH and Cancer

One of the key targets of HIF-1α activation is PDK. Kim et al. and our group have postulated that persistent activation of PDK and subsequent inhibition of pyruvate dehydrogenase (PDH) may be responsible for the "Warburg effect" in cancer cells.[7,78] PDH catalyses the irreversible oxidation of pyruvate, thus yielding acetyl CoA and CO_2, which then enter the TCA cycle and permit mitochondrial production of the electron donors NADH and FADH. PDH is thus a key enzyme controlling the rate of oxidative glycolysis. PDH is tightly controlled by the opposing effects of specific inhibitors (PDKs) and activators (PDH phosphatases). The PDH multi enzyme complex consists of multiple copies of three catalytic subunits, E1 (pyruvate decarboxylase), E2 (dihydrolipoamide acetyltransferase) and E3 (dihydrolipoamide dehydrogenase) in conjunction with the E3 binding protein. Phosphorylation of any of PDH's 3 regulatory serines in E1

by PDK completely inhibits PDH.[82] Four distinct but homologous PDK isoenzymes exist (PDK1–4); however, each isoenzyme displays distinct regulatory properties and tissue distributions. The tissue distribution of the PDK isoforms is similar in rats and humans.[83] PDK is a key regulator of mitochondrial activity since it phosphorylates and inhibits pyruvate dehydrogenase (PDH), thereby slowing the Krebs' cycle and restricting production of reducing equivalents (NADH, FADH) required to donate electrons to the ETC. This "inflow" obstruction may decrease respiration and reduce mitochondrial electron flux. This reduction in mitochondrial electron flux in turn decreases the associated the leak of superoxide which normally occurs in proportion to PO_2 as a result of the 3% of electron flux that is uncoupled.[7,8,84] The net effect of PDK activation is reduced oxidative metabolism and impairment of normoxic electron flux, which reduces mitochondrial ROS production.[37,70] In hypoxia, PDK's inhibition of the ETC is a beneficial, pro-survival mechanism, since ongoing electron transport without oxygen would not generate ATP but would instead cause detrimental mitochondrial superoxide formation by ETC autooxidation and overwhelm the superoxide detoxifying enzyme SOD2. However, even when O_2 is available, pathological activation of PDK can occur and suppress physiological O_2^- and H_2O_2 production in the mitochondria, thus creating a *pseudohypoxic state*, which allows cells to proliferate and prevents their removal by apoptosis. Kim et al. were able to show the central role of PDK as a cellular basis for the "Warburg effect" in P493–6 Burkitt's lymphoma cells, by demonstrating that PDK1 inhibition induces apoptosis in these malignant cells.[78] This is consistent with our demonstration that the PDK inhibitor, dichloroacetate, induces apoptosis and decreases proliferation in three human cancers, studied in a xenotransplantation model.[7] Using siRNA we have demonstrated that knocking down the PDK2 isoform of PDK in cancer cells depolarizes mitochondria and increases ROS production.[7]

PDK activation and impaired mitochondrial ROS production is also a common feature of FHR PAH.[8] Supporting the causal role of PDK activation in PAH is the observation that the PDK inhibitor dichloroacetate can regress all forms of experimental PAH tested to date (chronic hypoxic PHT, monocrotaline PAH and FHR PAH).[8] At effective doses (0.75 g/L of drinking water) dichloroacetate has no overt toxicity over 1–2 months of study.[8] While dichloroacetate inhibits all PDK isoforms, AZD7545 and the other new synthetic PDK inhibitors are selective for PDK2 (IC_{50} 6.4 ± 2.2 nM), with lesser effects on PDK1 (IC_{50} 36.8 ± 18 nM). In contrast, dichloroacetate's IC_{50} for PDK2 is only 200 μm.[83,85] Like dichloroacetate, new PDK2 selective inhibitors are very effective in activating PDH in vivo. However, there are tissue variations in PDH activation and little work has been done on the lung. Dichloroacetate not only restores mitochondrial ROS production, but also eliminates normoxic HIF-1α activation (as evidenced by loss of the nuclear localization of HIF-1α)[8]. This suggests that while HIF-1α can induce PDK expression, the converse is also true: PDK inhibition inactivates HIF-1α[8] and points to a feedback mechanism between HIF-1α and PDK, which based on our data is mediated by mitochondrial production of H_2O_2 (Fig. 11.1).

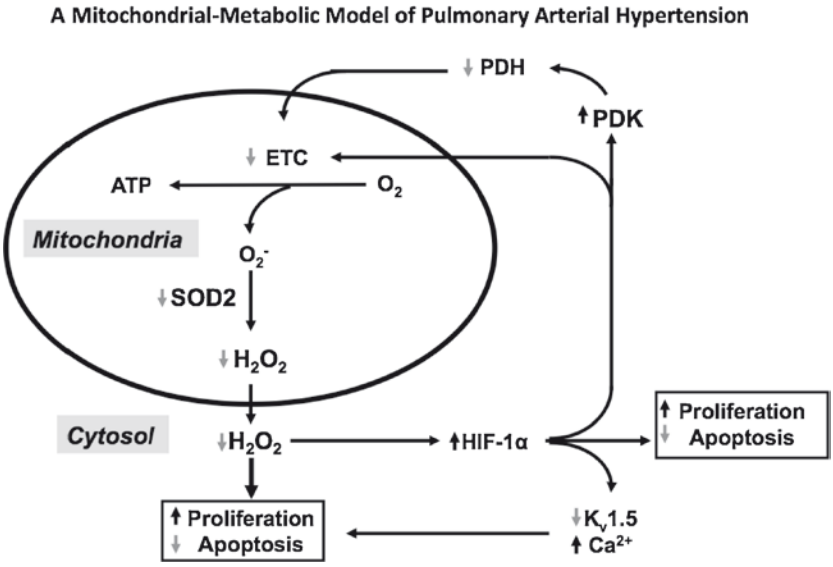

Fig. 11.1 Key aspects of the smooth muscle cell metabolic dysregulation in PAH. In aerobic metabolism, mitochondria are active producers of ATP via the electron transport chain (ETC), which allows electron donors (mitochondrial NADH and FADH) produced by the TCA (Krebs') cycle pass electrons down a redox-potential gradient in the ETC to molecular O_2. Side reactions of molecular O_2, accounting for ~3% of net electron flux, create the reactive oxygen species superoxide (O_2^-) in proportion to PO_2. SOD2 rapidly converts superoxide anion to H_2O_2, which serves as a signaling molecule, since it is less toxic than O_2^- and can diffuse out of the mitochondria due to its neutral charge. At physiological and supra-physiological levels H_2O_2 reduces cell proliferation and promotes apoptosis by either directly acting on the cell cycle or by inhibiting HIF-1α, which then inhibits PDK. This latter enzyme modulates PDH, which in turn enables pyruvate to enter the TCA cycle and aerobic metabolism to progress. In PAH, this balance is perturbed, since SOD2 activity and downstream H_2O_2 production are reduced. Decreased H_2O_2 production activates HIF-1α and reduces mitochondrial activity both by directly downregulating ETC enzymes as well as inhibiting PDH by activating PDK. Downregulation of mitochondrial activity further lowers mitochondrial O_2^- and H_2O_2 thus forming a feedback loop. This feedback loop and the abnormalities found in PAH are shown with arrows indicating the direction of change in activity of each element. The key pathology that contributes to vascular remodeling and PAH is the pro-proliferative and anti-apoptotic effect of this feedback loop. This is in part mediated by HIF-1α dependent and independent mechanisms, which can involve increases in $K_v1.5$ channels and higher cytosolic calcium levels

6 The Vicious Cycle of Metabolic Dysregulation in PAH

The mechanisms involved in the metabolic axis model of PAH that we have described offer numerous targets for the development of novel therapies in patients suffering from PAH. We propose that downregulation of the mitochondrial SOD2 in FHR decreases mitochondrial H_2O_2, production. Due to the neutral nature of the molecule, mitochondrial H_2O_2 can diffuse out of the mitochondria and therefore reduction of mitochondrial H_2O_2 also results in lower cytosolic levels of H_2O_2. This

not only has effects on possible direct biological targets of H_2O_2, but also activates the transcription factor HIF-1α. HIF-1α activation then enhances cell survival/proliferation and also increases PDK expression, which ultimately results in a vicious cycle as PDK inhibits oxidative metabolism and thus mitochondrial O_2^- and mitochondrial H_2O_2. While our unpublished data point to epigenetic silencing of SOD2 and reduction in mitochondrial H_2O_2 production as the initial trigger for this vicious cycle in PAH, the mitochondrial O_2^--SOD2-H_2O_2-HIF-1α-PDK-O_2^- feedback loop may also be initiated or exacerbated by additional triggers acting on elements of this loop (Fig. 11.1). Each of these abnormalities contributes to the abnormal *mitochondria-SOD2-ROS-HIF-PDK* pathway that we have described in human PAH and FHR PAH[8] as well as human cancer.[7]

7 Integrating Other Theories of PAH with the Mitochondrial–Metabolic Model

We acknowledge that many theories and models exist for the etiology of PAH and our focus in this chapter on *mitochondrial–metabolic* pathway does not imply that it is the only or the most important cause of PAH. The proliferation/apoptosis imbalance in PAH likely results from several, intersecting and reinforcing abnormalities that, including BMPR2 mutations,[29] de novo expression of the anti-apoptotic protein survivin[22,28] increased expression/activity of the SERT[31,32] and K_v1.5 downregulation.[8,26,36,37] Likewise, while we focus on a disorder that disturbs proliferation, we acknowledge the importance of endothelial dysfunction due to excess levels of endothelin and insufficient nitric oxide[19] or excessive rho-kinase mediated vasoconstriction.[12] In light of the high morbidity and mortality of PAH and our relatively limited armamentarium to help patients with this terminal disease it is critical that all avenues pointing to novel therapies be pursued. The mitochondrial-metabolic model offers both an explanation of the disease etiology based on numerous published studies as well as novel therapeutic avenues that can be used clinically in a very timely fashion, thus making this a very attractive approach.

8 Summary

Recognition of the central and interrelated roles of SOD2 downregulation, HIF-1α and PDK activation offers many promising therapeutic targets in PAH, including SOD2 replacement therapy, PDK inhibition and HIF-1α inhibition. The translational potential of this hypothesis is strengthened by the availability of drugs that are in clinical use to treat other human diseases, such as the PDK inhibitor dichloroacetate, or pharmacological agents used to act on epigenetic processes such as the DNA methyltransferase inhibitor 5-azacytidine. Dichloroacetate is used to treat

lactic acidosis related to mitochondrial diseases. Other PDK inhibitors are being tested in type II diabetes.[83] 5-Azacytidine (Decitabine®) is used to treat myeloproliferative disorders.[86] The current clinical use of such agents, which act on various components of the described metabolic dysregulation, in diseases such as diabetes or cancer facilitates testing them in clinical PAH trials.

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