Although traditionally pulmonary arterial hypertension (PAH) has been described as a disease exclusively of the pulmonary arteries, there is now evidence that PAH involves many other organs (Figure 1). For example, the hearts (right ventricle [RV]) of patients with PAH decompensate and fail at a much faster rate compared with what one would expect extrapolating from the left ventricle, suggesting that there may be mechanisms intrinsic to the RV that promote RV failure in PAH. Even patients with idiopathic PAH, without coexistent autoimmune disorders, have circulating immune cells that are activated, producing a strong inflammatory environment and potentially contributing to the malaise and weakness that patients with PAH have. Recent evidence suggests that, perhaps, their skeletal muscles (the target organs in the metabolic syndrome) have abnormalities similar to patients with diabetes mellitus, obesity, and...
metabolic syndrome, conditions also characterized by generalized inflammation. The serum of patients with PAH has increased levels of lipids and insulin although they may be neither obese nor diabetic. These muscle abnormalities may also contribute to the weakness these patients have. Are all these abnormalities simply resulting from a primary problem in the pulmonary arteries, perhaps related to the decreased cardiac output resulting from RV failure, or are they a part of a syndrome with multiorgan involvement?

Here, we propose a theory supporting the latter. We propose that there may be a global mitochondrial abnormality in many patients with PAH, which affects diverse tissues, including the pulmonary vascular cells, explaining the involvement of many organs in this disease. This suggests that treatments that may attack one of the foundations of the problem, ie, the mitochondria, might be more effective in terms of treating the patient as a whole and not just the pulmonary vessels. Another important implication of this theory is that the myriad molecular abnormalities that have been described in pulmonary vascular cells may have (at least many of them) a common denominator. After a cancer paradigm (where downstream signaling from diverse oncogenes converges to mitochondria, suppressing glucose oxidation with a secondary upregulation of cytoplasmic glycolysis, both contributing to the antiapoptotic and proproliferative phenotype of cancer cells), we propose that mitochondria can integrate abnormal signaling from several diverse causes and result in the PAH cellular phenotype as we know it today. This may simplify efforts to discover therapies because attacking the mitochondrial abnormalities may correct the effects of several primary molecular abnormalities, without having to attack every single one of them simultaneously.

**Non standard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>αKG</td>
<td>α-ketoglutarate</td>
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<tr>
<td>BMPR-II</td>
<td>bone morphogenic protein receptor II</td>
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<tr>
<td>DRP-1</td>
<td>dynamin-related protein 1</td>
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<tr>
<td>ER</td>
<td>endoplasmic reticulum</td>
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<tr>
<td>FDG</td>
<td>18F-labeled deoxyglucose</td>
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<tr>
<td>HiF-1α</td>
<td>hypoxia-inducible factor 1α</td>
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<td>MnSOD</td>
<td>manganese superoxide dismutase</td>
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<td>mROS</td>
<td>mitochondria-derived reactive O2 species</td>
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<td>MS</td>
<td>mass spectrometry</td>
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<tr>
<td>NFAT</td>
<td>nuclear factor of activated T cells</td>
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<td>PAEC</td>
<td>pulmonary artery endothelial cells</td>
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<td>PAH</td>
<td>pulmonary arterial hypertension</td>
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<td>PASMC</td>
<td>pulmonary artery smooth muscle cells</td>
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<td>PDH</td>
<td>pyruvate dehydrogenase</td>
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<td>PDK</td>
<td>pyruvate dehydrogenase kinase</td>
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<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>PPARγ</td>
<td>peroxisome proliferator–activated receptor γ</td>
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<td>RV</td>
<td>right ventricle</td>
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<td>TNFα</td>
<td>tumor necrosis factor α</td>
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<td>UCP</td>
<td>uncoupling protein</td>
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<td>VOC</td>
<td>volatile organic compound</td>
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**Figure 1.** Pulmonary arterial hypertension (PAH) involves many organs. Several organs are involved in PAH in addition to the resistance pulmonary arteries, which are characterized by an obliterative vascular remodeling (top left: smooth muscle actin staining of a small pulmonary artery from a patient with PAH; used with permission from Michelakis et al188). The right ventricle (RV) undergoes remodeling in response to increased afterload (top right: autopsy specimen form a patient with severe PAH showing hypertrophy and dilatation of the RV). Activated circulating (bone marrow–derived) immune cells infiltrate the lung parenchyma and significantly contribute to the pathology of the disease (bottom right: a remodeled pulmonary artery from a patient with PAH showing intense infiltration of mononuclear immune cells around and within the vascular wall). Reprinted from Stacher et al1 with permission of the publisher. Patients with PAH also have evidence of insulin resistance, resembling the metabolic syndrome. The skeletal muscle is a major target organ in the metabolic syndrome in humans. In the left lower corner, a biopsy of skeletal muscle from transgenic mice carrying a human bone morphogenic protein receptor II mutation is shown. The lipid droplets (arrow) are characteristic of insulin resistance. These signs appeared even before spontaneous pulmonary hypertension was detectable in these mice. Reprinted from West et al16 with permission from the publisher. Remarkably, as discussed in the text, all of these tissues have evidence of mitochondrial suppression and inhibited glucose oxidation.

**PAH: Pathology and Molecular Phenotypes**

**Pulmonary Arteries**

*Metabolic Remodeling in PAH Vascular Cells and Its Signaling Implications*

PAH is characterized by an obliterative vascular remodeling in the lungs. Pulmonary artery smooth muscle cells (PASMC), endothelial cells, fibroblasts, and myofibroblasts, at the peak of the disease, exhibit a proliferative and antiapoptotic phenotype that results in obliteration of the lumen by several of the recognized lesions, ie, intima and media hypertrophy and plexogenic arteriopathy. The pathology of the disease is also characterized by infiltration of activated inflammatory and
immune circulating cells. Of all the cells within the remodeled pulmonary arteries, the PASMC have been studied the most although the pulmonary artery endothelial cell (PAEC) and fibroblasts are increasingly being studied.

The molecular phenotype of PAH-PASMC persists even in vitro (where the potential primary triggers like circulating factors or paracrine signals are absent), suggesting that there may be positively reinforcing feedback loops that sustain the

<table>
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<th>Table. Molecular Abnormalities of Pulmonary Arterial Hypertension</th>
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ALK1 indicates activin A receptor type II-like 1; APJ, apelin receptor J; ApoE, apolipoprotein E; ATF6, activating transcription factor 6; BAX, B-cell CLL lymphoma 2-associated X protein; BMPR-II, bone morphogenic protein receptor II; c-Src, c-src avian sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog; ER, endoplasmic reticulum; GC, gas chromatography; GTPase, guanosine-5’- triphosphate hydrolase; HIF, hypoxia-inducible factor; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T cells; N0, nitric oxide; PDE5, phosphodiesterase 5; PDH, pyruvate dehydrogenase; PGC-1\(\alpha\), PPAR\(\gamma\) coactivator-1\(\alpha\); PPAR\(\gamma\), peroxisome proliferator–activated receptor \(\gamma\); RAGE, advanced glycosylation end product–specific receptor; RhoA, ras homolog family member A; ROCK, rho-associated, coiled-coil containing protein kinase 1; SMAD, mothers against decapentaplegic homolog; SOD2, manganese superoxide dismutase; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor β; UCP, uncoupling protein; and VIP, vasoactive intestinal peptide.
phenotype. The PAH-PASMC have many features of cancer cells, often exhibiting activated oncogenes or expressing cancer markers.\(^6\) Like in cancer, a long and diverse list of molecular abnormalities has been described in the past 15 years in PAH.\(^3,4\) With the unintended risk of missing some important findings, we list many of these molecular abnormalities in the Table. At first, one is impressed by the diversity of these mechanisms. Although many of these abnormalities have been successfully targeted in animal models in vivo, an important question arises: which of these are the most important, worthy of clinical translation? Practically, one can envision a combination therapy in the future with 2 or maybe 3 classes of drugs, like in systemic hypertension. But which of these should be left out? And does this not mean that the treatment would be incomplete because many abnormalities will remain active? We have recently proposed that this diversity is one of the main reasons that effective translation of animal work into humans is poor.\(^7\) We proposed that a comprehensive theory attempting to put many of these abnormalities under one roof might offer more effective therapies. The metabolic theory suggests that many of these factors directly or indirectly relate to the metabolic/mitochondrial phenotype of the disease (Table), which we describe below. In other words, such a theory predicts that a single therapy aiming to normalize a mitochondrial abnormality may be beneficial to a large number of abnormalities that, although on surface seem diverse, may essentially be a result of abnormal signaling downstream of mitochondria.

PAH-PASMCs are hyperproliferative and resistant to apoptosis, 2 properties that may be because of mitochondrial remodeling. Mitochondria are the metabolic sensors of the cell, and as such decide for both life (ATP production) and death (apoptosis).\(^6\) Mitochondria-dependent apoptosis maintains metabolic fuel efficiency and homeostasis, and its suppression offers a survival advantage to proliferating cells. PAH-PASMC mitochondria from several models and human tissues are hyperpolarized, have suppressed glucose oxidation and respiration, and have upregulated glycolysis.\(^6\) This normoxic glycolytic phenotype (the Warburg effect) is essentially identical to that of cancer cells.\(^5,7,8\)

There are several important downstream implications of this suppression of mitochondrial function that all result in characteristic features of PAH: (1) suppressed apoptosis, (2) suppressed signaling that affects several proproliferative downstream redox-sensitive extramitochondrial factors including transcription factors and plasmalemmal Kv channels, (3) increased availability of nonoxidized sugars, lipids, and amino acids for the building blocks of proliferating cells, and (4) potential signals to the nucleus regulating epigenetic mechanisms or even inducing inflammation via the induction of mitochondria-based inflammasomes. Here is how this happens:

**Suppressed apoptosis:** Proapoptotic mediators (like cytochrome C or apoptosis-inducing factor) efflux into the cytoplasm from the mitochondria through the mitochondrial transition pore, a voltage- and redox-sensitive megachannel. Increase in the mitochondrial membrane potential promotes closing of this channel and thus establishes a state of relative resistance to apoptosis.\(^7,9,10\) PASMC from many animal models and human tissues have hyperpolarized mitochondria like cancer cells.\(^6,7,9,11\) The generation of mitochondrial membrane potential is regulated by several mechanisms, and it is closely linked to the influx of carbons (carbohydrates, lipids) that enter mitochondria for oxidation. There is suppression of carbohydrates entry in mitochondria in PAH (and cancer), largely because the key enzyme regulating the influx of pyruvate (the product of glycolysis in the cytoplasm) into the mitochondria where it becomes acetyl-CoA and feeds the Krebs cycle (ie, pyruvate dehydrogenase, PDH) is inhibited.\(^6,12,13\) This results in suppression of glucose oxidation. The decrease in the efficiency of ATP production (2 molecules of ATP produced per glucose molecule in the cytoplasm from glycolysis versus 36 in the mitochondria from glucose oxidation) triggers several mechanisms to increase glucose entry and upregulate glycolysis. As part of this switch to glycolysis, on both PAH and cancer, glycogen synthase kinase 3\(\beta\) is activated and translocates from the cytoplasm to the mitochondrial outer membrane, where it binds and inhibits the voltage-dependent anion channel, a component of the mitochondrial transition pore.\(^6,14,15\) This traps anions in the mitochondria, increasing the membrane potential.

**Increased mROS** causes plasmalemmal depolarization, resulting in increased influx of Ca\(^{2+}\), which promotes constriction and proliferation.\(^16\) H\(_2\)O\(_2\) can also regulate redox-sensitive transcription factors. For example, the inhibition of Kv channels (activated by increased mROS and inhibited by decreased mROS) causes plasmalemmal depolarization, resulting in increased influx of Ca\(^{2+}\), which promotes constriction and proliferation.\(^16\) H\(_2\)O\(_2\) can also regulate redox-sensitive components of the transcription machinery of the proproliferative hypoxia-inducible factor 1\(\alpha\) (HIF-1\(\alpha\)).\(^7,17\) Similarly,
the Krebs cycle-derived α-ketoglutarate (αKG) can leak into the cytoplasm and regulate HIF-1α because it is a required co-factor for the prolyl-hydroxylases that destabilize HIF-1α.87,88 Thus, the primary changes in mitochondrial function occurring in cancer cells and idiopathic PAH-PASMCs decreases mROS and αKG production, resulting in HIF-1α stabilization (Figure 2) and activation, even in the absence of hypoxia, a well-described event in PAH.87,89 Although the biology of the PAH-PASMC is different than that of a normal PASMC, it is important to note that controversy exists on how hypoxia regulates mROS versus extramitochondrial reactive O2 species in normal PASMC. This controversy likely relates not only to the complexity of redox biochemistry and its dynamic nature but also to the many different techniques and models used by different groups.90–92

Nevertheless, HIF-1α can feedback and inhibit mitochondrial function by increasing the expression of pyruvate dehydrogenase kinase (PDK) an inhibitor of PDH, thereby sustaining it own activation.93 The increased cytoplasmic Ca2+ causes activation of the master transcription factor NFAT (a well-described feature of PAH)22,66,69,74 by promoting its entry to the nucleus. The switch toward glycolysis that follows mito-chondrial suppression also activates the enzyme glycogen synthase kinase 3β, which prevents the exit of NFAT from the nucleus promoting its activation.66,69,82 NFAT inhibits the transcription of many mitochondrial proteins and the expression of Kv channels.99 Thus, 2 important transcription factors that play a critical role in PAH participate in sustained activating feedback loops, which may explain why isolated PASMC maintain the same phenotype when cultured out of the PAH environment and why it is so difficult to reverse many molecular abnormalities in PAH.

Citrate, αKG, and epigenetic mechanisms: These diffusible small molecule products of the Krebs cycle can regulate both methylation (αKG) and acetylation (citrate) of nuclear histones.57,58 For example, citrate is considered to be the only known source of acetyl-CoA in the nucleus. Because acetyl-CoA is impermeable to membranes, citrate that leaks out of the mitochondria can enter the nucleus where in the presence of ATP-citrate lyase can provide acetyl-CoA for histone acetylation.57 Although not studied in detail, a role of histone deacetylase inhibitors as potential PAH therapies has been proposed.99 The benefits of these drugs suggest that histone acetylation is decreased in PAH-PASMC, which is compatible with the decreased production of citrate from the Krebs cycle (although it is possible that citrate could also be synthesized in the cytoplasm by the glutamine pathway, a mechanism established in cancer but not adequately studied in PAH).5

Inflammamomes: Although unexplored in PAH, we now know that mitochondria can induce inflammasomes, multiprotein complexes engaged by the nucleotide-binding oligomerization domain (NOD)-like family of cytoplasmic receptors. NOD-like family of cytoplasmic receptors continuously monitors the cytosol and on detection of cellular stress oligomerizes and exposes its effector domain for interaction with the adaptor apoptosis-associated speck-like protein, which in turn recruits procaspase-1. Pro-caspase-1 clustering leads to its activation via auto-processing, and active caspase-1 proteolytically cleaves a variety of cytoplasmic targets, including interleukin-1β (which is increased in PAH).94 After an activation cascade, several other
inflammatory cytokines are subsequently activated, also known to be increased in PAH (as discussed in another chapter of this compendium). The NOD-like family of cytoplasmic receptors inflammasome is activated by high glucose levels in β cells in the pancreas, leading to cleavage and secretion of interleukin-1β. In addition to cleaving interleukin-1β, caspase-1 also targets several key glycolytic enzymes, such as aldolase and pyruvate kinase, suggesting that the NOD-like family of cytoplasmic receptors inflammasome reciprocally regulates cellular metabolism under stress conditions.

Similar glycolytic phenotypes, for example, increased uptake of glucose and increased production of lactic acid, have also been described in PAH-PAEC. After the cancer paradigm, this is likely a result of a primary suppression of glucose oxidation. These mechanisms yet have not been studied in fibroblasts, to the best of our knowledge. Last, the many ways that the cancer stroma can regulate metabolic pathways in the cancer cells have not been studied in PAH, where the interstitial matrix nevertheless plays an important role in overall tissue remodeling.

In summary, many aspects of the PAH cellular phenotype (eg, hyperpolarized mitochondria, decreased mROS, activated HIF-1α and NFAT, inhibited Kv channels, increased cytoplasmic calcium, suppressed histone acetylation, and activated inflammation) can be potentially explained by an inhibition of mitochondrial oxidative phosphorylation (and specifically glucose oxidation). What is the cause of this inhibition? Below we describe several intramitochondrial and extramitochondrial causes that have been described in PAH.

**Causes of the Metabolic Remodeling in PAH**

**Vascular Cells**

Extramitochondrial causes: Regulation of PDH. A major regulatory mechanism for PDH is its tonic inhibition by PDK with which it forms a complex. There are 4 isoforms of PDK, with PDK1 and 2 being ubiquitously expressed, PDK3 being...
expressed in the testes, and PDK4 being inducible mostly in muscle under metabolic stress. The expression of PDK1 is induced by HIF-1α, and its activity can be enhanced by tyrosine kinase phosphorylation (thus both conditions inhibiting PDH); both HIF-1α and tyrosine kinase axis activation are well-described features of animal and human PAH. The importance of PDH in PAH and cancer is supported by the fact that the small molecule inhibitor of PDK dichloroacetate activates PDH, reversing the glycolytic shift and the resistance to apoptosis of PASMC and both prevents and reverses PAH in several models of PAH in vivo (Figure 3A; as well as reverses cancer growth in several cancer models and in a small mechanistic trial of dichloroacetate in patients with glioblastoma). The dichloroacetate-dependent induction of PASMC apoptosis in PAH vessels causes an effective reversal of established pulmonary vascular remodeling, and this seems to be specific to the diseased pulmonary circulation. Dichloroacetate increases survival in rats with PAH and its ability to also improve RV contractility further enhances its therapeutic potential.

Another way that PDH activity can be inhibited is by the activation of fatty acid oxidation. The Randle cycle ensures that only 1 of the 2 mechanisms, ie, glucose versus fatty acid oxidation, can be used as a primary fuel source at any given time, thus inhibition of fatty acid oxidation enhances PDH activity. Drugs or molecular mechanisms that decrease the activity of the fatty acid oxidation regulator malonyl-CoA decarboxylase enhance PDH activity, preventing the mitochondrial remodeling under PAH stimuli. Thus, trimetazidine and knockout of malonyl-CoA decarboxylase cause reversal and prevention of PAH in rodent models in vivo.

Endoplasmic reticulum (ER) stress. PDH and several other mitochondrial enzymes (eg, αKGD-dehydrogenase and isocitrate dehydrogenase) are calcium-dependent. Thus, a decrease in mitochondrial Ca2+ inhibits PDH. We now know that ER stress can decrease mitochondrial calcium, by causing functional disruption of the ER-mitochondria unit. This is because the ER is the major calcium supplier of mitochondria. Expression of the reticulon protein Nogo, under the ER stress-activated transcription factor 6 axis, alters the shape of the ER, disrupting the functional mito-ER unit at specialized contact points of the 2 organelles, where the exchange of calcium and lipids occurs (Figure 4). PASMC lacking Nogo do not develop the PAH phenotype under hypoxia in vitro, and Nogo knockout mice are resistant to the development of chronic hypoxia–induced pulmonary hypertension. ER stress is a result of many known triggers of PAH including bone morphogenic protein receptor II (BMPR-II) mutations (which can result in accumulation of unfolded protein aggregates), viral infections (like human immunodeficiency virus and herpes simplex virus), upregulation of Notch3, hypoxia, or inflammation. Increased Endothelin-1 signaling, a well-studied pathway in PAH, was also recently shown to induce ER stress.

Inhibition of ER stress by the small molecular chemical chaperone phenylbutyrate reverses the decrease in mitochondrial calcium and the PDH inhibition and normalizes the cellular phenotype of PAH, whereas it prevents and reverses PAH in rodent models. ER stress in PAH-PASMCs has also been linked to increased production of cytokines like interleukin-6 and chemokine (C-C motif) ligand 2/ mast cell proteinase-1.
which in turn promote the activation and recruitment of macrophages in the lung. Another inhibitor of ER stress, salubrinal, also reverses monocrotaline PAH in a PERK (protein kinase RNA-like endoplasmic reticulum kinase)-dependent manner, and this was also associated with decrease recruitment of macrophages in the lung. ER stress is a feature of PAH-PAEC as well. In fact, a structurally abnormal ER (suggesting ER stress) in PAEC was prominent in the early classic descriptions of PAH pathology by Smith and Heath in 1979.

Additional factors suppressing mitochondrial function in PAH. There are many other examples in which additional well-characterized pathways in PAH can, in addition to their primary effect, lead to mitochondrial suppression, including signal transducer and activator of transcription 3, suppressed BMPR-II signalling and peroxisome proliferator–activated receptor γ (PPARγ) signaling. For example, signal transducer and activator of transcription 3 can downregulate BMPR-II/PPARγ and activate NFAT22 (which, as discussed, decreases the expression of many mitochondrial enzymes and members of the bcl-2 family); the BMPR-II axis inhibition as discussed above can cause ER stress (in the case of mutated dysfunctional BMPR-II receptors that disturb cellular trafficking) or inhibit PPARγ signaling, which in turn inhibits mitochondrial biogenesis; PPARγ activators have shown promise as potential therapies of PAH in animal models. PPARγ also regulates the expression of Apelin, which has been shown to increase mitochondrial biogenesis, improve mitochondrial oxidative capacity, and enhance insulin-stimulated glucose uptake.

Mitochondrial function can also be suppressed by dysregulation of the processes that regulate the mitochondrial network in the cytoplasm (fission/fusion) and mitochondria numbers (mitogenesis and mitophagy), which optimize energy and signaling production particularly in critical microdomains in the cells, in response to stressful stimuli. There is evidence that both processes, in response to extramitochondrial triggers, are disturbed in PAH:

Fission/fusion. The fusion machinery consists of the dynamin-like proteins mitofusin 1 and 2 that promote the fusion of the outer mitochondrial membrane and optic atrophy 1, which is involved in the sequential fusion of the inner mitochondrial membranes. Mitochondrial fission, however, mainly results from the dynamin-related protein 1 (DRP-1), a large GTPase assembled into oligomeric rings around the outer mitochondrial membrane. The maintenance of mitochondrial network and shape depends on a constant equilibrium between fusion and fission events, and alterations of this balance can disrupt mitochondrial signaling and regulation of cell cycle progression or apoptosis. The mitosis-initiating kinase B1-CDK1 phosphorylates DRP-1 at serine 616, leading to fission and regulating an equitable distribution of mitochondria to daughter cells on cell division.

The mitochondrial suppression of PAH-PASMC has recently been linked to increased fission and increased activity of DRP-1. Suppression of DRP-1 by siRNA or the inhibitor Mdivi-1 prevented fission, decreased proliferation, and arrested PAH-PASMCs at the G2/M interphase. These antiproliferative effects were beneficial in several models of rodent PAH, and similar mechanisms were described in various cancers. Interestingly, Mdivi-1 has also been shown to improve the muscle insulin sensitivity in obese mice, suggesting, first, a role of mitochondrial dynamics in insulin resistance in skeletal muscle and, second, that Mdivi-1 may be beneficial on several aspects of the mitochondrial abnormalities seen in PAH in several organs.

Whether inhibition of fission leads to activation or inhibition of apoptosis is not clear because some controversy exists. For example, some but not all studies show that inhibition of DRP-1 may suppress apoptosis, which may seem to be in conflict with its beneficial effects in PAH. More studies are needed to clarify this issue because the regulation of apoptosis in PAH is complex and depends on the timing of the disease model and the cellular compartments (e.g., early in PAH, there is increased apoptosis of endothelial cells although later on, there is decreased apoptosis of both PAEC and PASMC).

Decreased fusion and expression of mitofusin 2 and its transcriptional coactivator PPARγ, coactivator-1α, were also recently shown to contribute to mitochondrial fragmentation and a proliferation-apoptosis imbalance in human and rodent PAH. Augmenting mitofusin 2 with replacement gene therapy showed benefit in human PAH-PASMC in vitro and rodent PAH in vivo, decreasing proliferation and promoting apoptosis.

Mitogenesis/mitophagy. Mitochondria are continuously renewed by a physiological balance between biogenesis and mitophagy, which selectively degrades mitochondria, particularly those that produce high levels of mROS. Interestingly, the loss of fusion while fission is active (through optic atrophy 1 overexpression, fission 1 [mitochondrial outer membrane] homolog RNAs, and DRP-1 dominant negative expression) has been associated with reduced levels of mitophagy, suggesting that there may be an accumulation of dysfunctional mitochondria in such states. The role of mitophagy in PAH has not yet been fully investigated.

In summary, extramitochondrial factors like the transcription factors, signal transducer and activator of transcription 3, NFAT, PPARγ, PPARγ coactivator-1α, or dysregulated BMPR-II signaling, can all decrease the gene expression of mitochondrial factors and disrupt mitochondrial dynamics, contributing to an overall suppression of mitochondrial function in PAH vascular cells. In addition, inflammation, via the induction of ER stress, or direct inhibition of mitochondrial enzymes (like the tumor necrosis factor α (TNFα)-induced inhibition of PDH), also results in suppressed mitochondrial function in PAH vessels. These data are summarized in Figure 5.

Intramitochondrial causes: Uncoupling protein (UCP) 2. The mitochondrial protein UCP2, despite its name, does not have significant mitochondrial uncoupling properties, but rather functions as a Ca2+ uniporter, facilitating Ca2+ entry from the ER into the mitochondria. UCPs belong to the superfamily of mitochondrial ion transporters and are embedded in the inner mitochondrial membrane. With the exception of the prototypical UCP1, which causes a primary uncoupling of the mitochondria by functioning as a H+ channel, the effects of other UCPs in uncoupling have been challenged. UCP2 is expressed in many tissues including the lungs and inflammatory cells. Ucp2KO-PASMCs have lower mitochondrial
Ca\textsuperscript{2+} levels, mitochondrial hyperpolarization, lower activity of Ca\textsuperscript{2+}-sensitive mitochondrial enzymes (like PDH), and resistance to apoptosis, compared with PASMC from the wild-type controls.\textsuperscript{76,77} Normoxic Ucp2KO-mice spontaneously develop pulmonary vascular remodeling and pulmonary hypertension\textsuperscript{76,77} (Figure 3B). These data showed for the first time
that an abnormality in a protein intrinsic to mitochondria is sufficient to induce PAH in the absence of other triggers. It is intriguing that loss of UCP2 has also been linked to predisposition to cancer in mice.136 In addition, UCP2 has been linked to the metabolic syndrome.137 Polymorphisms in the human UCP2 gene (that result in decreased expression/activity of the protein) have been associated with several diseases. The UCP2 G(-866)A (rs659366) polymorphism located in the promoter region of the gene, is associated with lower mRNA expression levels.138–142 This -866 G allele has been associated with increased risks of chronic inflammatory diseases143 and greater susceptibilities to autoimmune diseases138 and cardiovascular diseases,144–146 including the metabolic syndrome.137

Iron homeostasis. Another intriguing but less studied observation is that patients with PAH show evidence of anemia and dysfunctional iron homeostasis.61,62 Iron is a critical substrate for mitochondria as Fe-S clusters (exclusively formed in mitochondria) are essential components of electron transport chain complexes and enzymes like succinate dehydrogenase or aconitate.147 A general mitochondrial dysfunction, which includes iron-overloaded mitochondria, characterizes the increasing list of human diseases that are caused by mutations that disrupt Fe-S clusters and iron homeostasis in mitochondria.148 The emerging evidence suggests that PAH may belong to this list of diseases.

MnSOD. Recent evidence suggests that the SOD2 gene that encodes for the mitochondrial protein MnSOD is hypermethylated, resulting in decreased expression of MnSOD, contributing to the observed decrease in H$_2$O$_2$ in PAH-PASMCs.65 Treatment with the DNA methyltransferase inhibitor 5-aza-2’-deoxycytidine restored MnSOD expression, decreased proliferation, and increased apoptosis in PAH-PASMCs.65 It is interesting that this epigenetic regulation seems to be because of a tissue-specific upregulation of DNA methyltransferase 1 and 3 in pulmonary but not systemic arteries.65

Taken together, these data suggest that whether there is a primary nonmitochondrial signal with secondary effects on the mitochondria or a primary intramitochondrial abnormality, eventually mitochondrial function is suppressed in PAH. These data are summarized in Figure 5. Thus, mitochondrial suppression may either directly cause PAH or facilitate its progression.

**Metabolic Changes in the PAH RV**

RV failure is the primary cause of morbidity and mortality in patients with PAH.149–151 As an early and short adaptive response to increased afterload, the RV initially undergoes hypertrophy. With persistent increase in afterload, myocardial apoptosis, excessive fibrosis, or increased expression of noncontractile proteins, all contribute to decreased RV systolic function and then to dilatation and failure. A metabolic switch from mitochondrial oxidative phosphorylation to glycolysis152 has been described in the compensated phase of RV hypertrophy (ie, while cardiac output is maintained).153 RV mitochondria become more hyperpolarized,154 suppressing mitochondria-dependent apoptosis, perhaps as a response to the stress in an attempt to preserve the cardiomyocytes. There is also evidence of decreased mROS production and activation of HIF-1α and NFAT in the compensated phase of RV hypertrophy, pointing to an intriguing similarity to the biology of cardiomyocytes and that of cancer cells and idiopathic PAH-PASMCs. In addition, the hypertrophied RV shows increased glucose uptake measured by positron emission tomography, similar to the PAH pulmonary vessels. This similarity is emphasized in Figure 2. The decrease in mROS may also be seen as an attempt to minimize additional sources of stress in a myocardium that struggles to hypertrophy to maintain cardiac output. The HIF-1α activation promotes angiogenesis because increased blood/oxygen supply is needed to support the growing myocardial mass. Increased levels of mitophagy have been suggested in the RV, in a pulmonary artery constriction model of RV hypertrophy, with increased expression of autophagy/mitophagy markers Light Chain 3A/B and p62,154 whereas mitochondrial biogenesis maybe impaired because of the decreased expression of PPARγ coactivator-1α.155

However, recent evidence suggests that this potentially adaptive switch seems to be reversed on entrance into the decompensated phase of RV function (where the cardiac output decreases and mortality increases sharply), suggesting that the mitochondrial suppression maybe a beneficial and reversible event in RV hypertrophy. The increase in glucose uptake, the activation of HIF-1α, and the mitochondrial hyperpolarization are lost at this point.64 Because the mitochondria start to paraadoxically function again, they resemble mitochondria from the normal RV; only now, the afterload remains high, and the myocardium is unable to defend. The loss of the angiogenic program causes a loss of microvessels, contributing to ischemia, and the loss of mitochondrial hyperpolarization may facilitate the apoptosis and thus the loss of myocardial cells that characterizes myocardial decompensation and failure.153 The trigger for the loss of this adaptive molecular and metabolic program is unknown. Because this entry to a decompensation phase occurs much earlier that it does in the left ventricle facing similar levels of increased afterload, it is likely that it is associated with a mechanism intrinsic to and specific to the RV because the 2 heart chambers have many and important differences in their embryology,156 structure, and molecular physiology.140

**Metabolic Changes in Activated Inflammatory Cells**

Inflammation is strongly associated with the metabolic disturbances seen in diabetes mellitus and insulin resistance157; the similar and unexplained resistance to insulin seen in patients with PAH may be worsened by the generalized inflammatory environment.118,158 Inflammation characterizes many animal PAH models and is an increasingly recognized feature of clinical PAH,1,159,160 also reviewed elsewhere in this compendium. The inflammatory milieu in PAH is composed of resident and recruited macrophages, dendritic cells, T and B cells, and mast cells.161,162 Intriguingly, many immune cells become highly glycolytic on activation because of a suppressed glucose oxidation (ie, they have the exact same mitochondrial remodeling seen in cancer cells and in PAH vascular cells), suggesting that these cells may also be responsive to mitochondria-targeting therapies.163 Furthermore, many of the cytokines produced from these immune cells can also have direct effects on pulmonary artery mitochondrial function. For example, TNFα can directly inhibit PDH activity in PASMC,
resulting in activation of NFAT and HIF-1α, apoptosis resistance, and enhanced proliferation. Inhibition of TNFα by etanercept (a drug used in patients with rheumatoid arthritis) normalized the metabolic phenotype of PAH in vitro and reversed established monocrotaline-induced PAH in vivo. The TNFα antibody infliximab reduced pulmonary pressures and improved the quality of life in a patient with severe PAH secondary to advanced scleroderma. These data suggest that the metabolic and inflammatory theories in PAH overlap and may even potentiate each other.

**Metabolic Changes in the Skeletal Muscle**

A high frequency of insulin resistance and a metabolic syndrome-like picture have been described in patients with PAH, even in the absence of obesity or diabetes mellitus. In fact, indices of insulin resistance in the serum of patients with PAH are associated with worse outcomes.

Patients with PAH have reduced mRNA expression of PPARγ in the lung (a ligand-activated nuclear receptor and transcription factor that regulates adipogenesis and glucose metabolism) and apolipoprotein E (a protective factor known to reduce circulating oxidized low-density lipoproteins). Deficiency of both PPARγ and apolipoprotein E has been linked to insulin resistance and the metabolic syndrome. There are signs of mitochondrial abnormalities in the skeletal muscle (a tissue that exhibits signs of insulin resistance in the metabolic syndrome) of PAH animals. PPARγ coactivator-1α, nuclear respiratory factor 1 and transcription factor A mitochondrial mRNA transcripts were found decreased as early as 2 weeks post monocrotaline injection in the gastrocnemius muscle of rats, ie, a time at which the pulmonary pressure is not significantly increased and the cardiac output has not started decreasing. It is still not clear, however, whether these metabolic abnormalities are causative or a consequence of PAH. Apolipoprotein E deficient (ApoE−/−) mice that develop insulin resistance on a high-fat diet also develop PAH, RV hypertrophy, and pulmonary vascular remodeling. This response is attenuated or reversed by the PPARγ agonist rosiglitazone, showing that direct attenuation of insulin resistance, even in the absence of obesity or diabetes mellitus, may even potentiate each other.

**Circulating Factors in the Metabolic Remodeling of PAH Tissues**

There are 2 possibilities for the explanation of the mitochondrial abnormalities seen in several organs in PAH. First is the presence of a generalized intramitochondrial abnormality (eg, a UCP2 loss-of-function polymorphism and a mutation in the Fe-S cluster formation) or a generalized extramitochondrial trigger that suppresses mitochondrial function (eg, suppression of BMPR-2 signaling and ER stress because of enhanced inflammation) in all organs. Second, in addition to circulating inflammatory cytokines that can suppress mitochondrial function globally, there may be additional, yet unidentified, circulating factors that can cause mitochondrial suppression in many organs. Recent work has suggested that mitochondrial dysfunction in 1 organ can cause the production of mitokines that can circulate and disseminate mitochondrial suppression signals in remote organs. Durieux et al engineered transgenic worms, in which the gene cco-1 (worm homologue gene encoding for a subunit of the cytochrome c oxidase/cytochrome c oxidase 4) was disabled in a tissue-specific manner in the brain. They then found evidence of mitochondrial dysfunction in remote organs like the intestine. Although the nature of this signal remains unknown, it seems that it involves the mitochondrial unfolded protein response. The authors suggested that a diffusible molecule, a mitokine, is released from certain tissues, broadcasting a mitochondrial signal to remote target tissues. It is tempting to speculate that mitokines can be circulating between different organs in PAH as well. This area of research, yet unexplored in PAH, may eventually prove to be important for diagnosis or therapy.

Additional examples of mitochondria-produced diffusible factors that can circulate in the blood and affect several organs include the Krebs cycle’s products succinate and αKG. These diffusible small molecules are also measurable in the blood of patients with vascular disease, suggesting that they may exert systemic effects. Although not tested in PAH, it was recently shown that they can bind to G protein-coupled receptors in vascular cells and regulate the angiotsenin axis in systemic vascular disease models.

**Implications of the Metabolic Theory in Therapeutic and Biomarker Discovery Programs**

**Mitochondria-Targeting Therapies for PAH**

Several agents that hold promise in the preclinical treatment of PAH were discussed above: PDK inhibitors like dichloroacetate, malonyl-CoA decarboxylase inhibitors like trimetazidine, PPARγ activators like pioglitazone or rosiglitazone, NFAT inhibitors like cyclosporine, TNFα antagonists like etanercept, ER stress inhibitors like phenylbutyrate, mitochondrial fission inhibitors like Mdivi-1, and MnSOD activators like MnTBAP or the DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine, which increases MnSOD expression. Their site of action and how this relates to the metabolic theory of PAH is shown in Figure 5. The preclinical evidence for some of these metabolic modulators has been strong, as in the case of dichloroacetate discussed earlier. The fact that dichloroacetate and many of these drugs have been studied in humans with other conditions increases their translational potential. A challenge will be the fact that most of these drugs are generic, imposing funding challenges for clinical translation. Nevertheless, several investigator-driven clinical trials are now ongoing with some of the drugs in this list. These include a trial assessing the effects of dichloroacetate treatment in patients with advanced PAH, currently ongoing at the University of Alberta (Edmonton, Canada) and Imperial College of Medicine (London, UK; clinical trials #NCT01083524); ongoing trials from Stanford University studying the effects of PPARγ activators and NFAT inhibitors in patients with PAH (clinical trials #NCT00825266.
Metabolic Biomarkers in PAH

Because all the discussed mitochondrial abnormalities alter the production of diffusible metabolites, their systematic detection and profiling can be used to evaluate mitochondrial activity. Current imaging methods can also study and follow large metabolic shifts, like the enhanced glycolysis and glucose uptake that follows the suppression of mitochondrial glucose oxidation. For example, 18F-labeled deoxyglucose (FDG) is a radiotracer used for positron emission tomography, and because of its analogy to glucose, FDG is taken up in cells via the glucose transporter-1 (Glut1). However, because of the lack of a 2'-hydroxyl group preventing further metabolism, FDG accumulates and can be visualized. FDG uptake is increased in glycolytic conditions like cancer. Increased FDG uptake has also been identified in the lungs of patients with idiopathic PAH,97,174 but the precise origin of the signal was unclear, the technique not permitting to clearly separate inhomogeneous signals from airway versus pulmonary artery, or from PASMCs versus PAECs.174 However, studies in animal models suggest that the origin of the signal is in the vasculature, correlating with histology, hemodynamics, and metabolism. Increased RV FDG uptake has also been described in patients175–177 and rats with PAH,103 and the signal was reduced by therapies. Because the biology of both the pulmonary vessels and the RV myocardium is important for the prognosis and treatment of patients with PAH, the ability of positron emission tomography imaging to potentially simultaneously image the metabolic shifts in both tissues (Figure 2) makes this technique attractive although its cost and availability may restrict its use as an end point in clinical trials for now.

The measurement of metabolites is starting to be used in PAH after the cancer paradigm. Several available technologies can be useful in this direction, including magnetic resonance spectroscopy, nuclear magnetic resonance (mostly 1H-nuclear magnetic resonance), or mass spectrometry (MS). These may be used in combination with separation methods (liquid chromatography–MS and gas chromatography) as used in cancer, which—as discussed—causes essentially identical to PAH metabolic shifts in the affected tissues.179 Nuclear magnetic resonance permits the simultaneous detection and quantification of multiple metabolites but lacks sensitivity.179 In comparison, MS is sensitive, selective, automated, and costs less.190,181 Although the field is in its infancy, proof-of-principle early publications using MS for systematic metabolic studies of animal PAH vascular tissues have confirmed the ability of this approach to detect the large-scale metabolic shifts that follow the suppression of mitochondrial function.182 Gas chromatography–MS has been used in the detection of volatile organic compounds in the exhaled air of patients with cancer.193,184 This breath-printing approach has been rapidly extended to several other diseases including cardiopulmonary diseases.185–187 The proximity of the resistance pulmonary vessels to the small airways suggests that the detection

A Patient Asks Questions...

I am fit and watch my diet but my triglycerides are high and I read that my lung blood vessels have the same metabolism to that of a tumor. What does this mean and how important is it for my condition?

Our understanding of pulmonary arterial hypertension (PAH) is rapidly changing. This is important because it may result in the discovery of better, more effective therapies with fewer side effects. We have now come to realize that the energy-producing units in the cells (ie, the mitochondria) have many more functions in addition to providing the energy that we need to live. Mitochondria produce many signals in the cell that can affect many of its functions, including the function of our genes themselves. We know that suppression of mitochondrial function promotes, for example, the rapid division and growth of cells. Surprisingly, cells with suppressed mitochondrial function grow faster, but they do not seem to have loss of energy because they are able to develop alternative sources of energy production. We know that PAH is caused at large by rapid growth of the cells in the wall of the lung blood vessels. Importantly, animal work shows that re-energizing the dysfunctional mitochondria of these cells inhibits their ability to grow fast and thus improves the lung blood vessel function and PAH. There are available drugs that can achieve this in animals (eg, the generic drug dichloroacetate), but despite the promising results in animals, they still have to be proven effective and safe in humans. Such trials are now ongoing and allow us to remain optimistic. Such drugs have also shown promise in animals and humans with cancer, another disease characterized by rapid growth of cells. Intriguingly, the mitochondria in both cancer cells and cells in the wall of the lung blood vessels in PAH are characterized by suppression of mitochondria, ie, they share similar metabolic profiles. This allows us to take many lessons from the research in cancer that is more intense than the research in PAH because cancer is a much more common disease.

There is evidence that this mitochondrial dysfunction is also present in many other tissues in a patient with PAH, involving the right heart chambers, blood cells, or the skeletal muscles. Studies in diseases like diabetes mellitus type II and the common metabolic syndrome (a condition common in overweight patients) are also characterized by mitochondrial dysfunction. Because in these diseases one finds increased levels of lipids or insulin in the blood, it is not surprising that we now know that this is also present in some patients with PAH. Only in patients with PAH, these are not because of diabetes mellitus or excessive weight but, perhaps, because of dysfunctional mitochondria. Although research is too young at this point to have practical implications, it may change the way we approach PAH, from a disease confined within the lung blood vessels to a disease that affects mitochondria in the whole body. This will help identifying better diagnostic tools and therapies.

For the case description, see introductory article by E.D. Michelakis, page 109.
of metabolic signatures in the breath of patients with PAH is theoretically possible and holds promise as a potential noninvasive metabolic biomarker.

Conclusion
The emerging metabolic theory of PAH proposes that several organs in animals and humans with PAH may share mitochondria-based metabolic abnormalities. This theory may facilitate our understanding of the disease because at least several molecular abnormalities that may be seen as diverse and isolated events may actually all be consequences of a primary mitochondrial defect. There is some evidence at least in mice models that these defects may be causative for PAH. It also seems that such defects may facilitate the progression of the disease because they promote proliferation and inflammation and suppress apoptosis. Thus, the many potential therapeutic targets and biomarkers that this theory reveals may open a new window in our approach to this complex disease.

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