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REVIEW

Metabolic modulation of cancer: a new frontier with great translational potential

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Abstract Metabolic oncology is an exciting new field in cancer research, offering a new window to cancer's molecular plasticity and promise for the development of effective, cancer-selective therapies and novel biomarkers. It is based on the realization that cancer's unique metabolism (known since Warburg's report in 1923) with suppression of mitochondrial glucose oxidation and upregulation of cytoplasmic glycolysis is not a secondary but a primary event, offering many growth advantages to cancer cells. Many mechanisms have been revealed, including growth factors, oncogenes, and mutations, all contributing to a suppression of mitochondria, similar to what takes place in hypoxia. This suppression leads to inhibition of mitochondria-driven apoptosis, promotes proliferation, and enhances angiogenesis and metastatic potential. A number of molecular tools and small molecules targeting metabolic enzymes, including pyruvate kinase, pyruvate dehydrogenase kinase, isocitrate dehydrogenase, and lactate dehydrogenase, have been developed, inhibiting cancer growth in vitro and in vivo in several cancer types. Several have already entered early-phase trials, a great translational success considering the young age of the field (less than 10 years). Here we review the mechanisms and effects of these metabolic modulators and the rationale for further development. This rapidly accumulating knowledge allows some optimism that this may prove to be a paradigm shift in the way we understand and treat cancer.

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Introduction

The majority of chemotherapeutic drugs for cancer inhibit pathways fundamental to the life of all cells, leading to adverse effects on healthy tissues [1]. In addition, traditional drug development in cancer has been focusing on a single molecular pathway, following the "one gene - one drug" approach. It became apparent however that most tumors are characterized by multiple molecular abnormalities so that when the therapies are offered even in combination of 2-3 drugs, the tumor eventually relapses. It is a rather rare example to find therapies that are effective and not toxic. This requires the identification of molecular abnormalities that are not only critical for the life of cancer—but not normal cells—but are also the dominant or the only molecular abnormality within the tumor. For example, this can be seen in certain leukemias or tumors where a mutation in the majority of cancer cells not only dominates their molecular phenotype but is also critical for their survival, explaining the great success of agents such as Gleevec for chronic myelogenous leukemia [2] or Herceptin for certain breast cancers [3]. Yet, most cancers, like glioblastoma for example, are characterized by several cellular phenotypes within each tumor and yet, in each of these cell types, there are several genetic and molecular abnormalities [4], obviously not susceptible to a single or even a combination of two to three drugs. Is it possible to identify a common denominator across all of these abnormalities that is not only critical for the survival of cancer cells but is also not present in normal cells? And to push the envelope even further, is it possible that this common denominator is also present in cancer stem cells, so that if targeted, tumor relapse would be limited as well [5]? In other words, is there an

Achilles' heel for such complex and molecularly plastic tumors? Recent work over the past 10 years suggests that such a common denominator, unique to cancer cells, may exist and be no other than the unique metabolism of cancer cells, first identified by Otto Warburg, more than 90 years ago [6]. Unfortunately, the work of Warburg, who was awarded the Nobel Prize for his work on metabolism, did not translate in cancer therapies for many decades as it was wrongly assumed that the unique cancer metabolism was a consequence, not a cause or contributing factor in cancer. We now know that if not causal, like in certain examples of mutations in key metabolic enzymes, this metabolic remodeling is a strong contributing or promoting factor as it promotes proliferation, resistance to apoptosis, and tumor angiogenesis, facilitating tumor growth and metastasis. Even better, tumor metabolism is typically different than that of normal cells. The development of "metabolic modulators" over the past 10 years has already entered early-phase trials. Although it sounds "too good to be true" that metabolism is the long sought Achilles' heel for cancer, a new field has already opened. Metabolic oncology is an exciting frontier in the battle against cancer, and this review focuses on the rationale for the development of metabolic modulators, concentrating on those that hold promise or already have entered human trials.

Cancer's abnormal metabolism and its molecular consequences

The cornerstone of metabolism in the cell is the mitochondrion. Traditional biochemistry taught us that mitochondria's main job is to generate heat and energy (ATP). We now know that although the most efficient means for ATP production lie within mitochondria, ATP can be adequately produced outside of mitochondria, through cytoplasmic glycolysis, at amounts enough to support the energy-hungry cancer cells. We also now know that mitochondria do much more: They produce diffusible mediators that can regulate multiple molecular pathways in the cell and even the nucleus. They can also induce apoptosis, an intriguing fact since the "provider of life" (i.e., ATP) can also be the provider of death for the cell. It was perhaps this "paradox" that did not allow mitochondria to be seen as targets for pro-apoptotic strategies until recently. Therefore, suppression of mitochondrial function can suppress apoptosis and alter cellular signaling toward a pro-proliferative phenotype as we discuss below. It is thus not a surprise that cancer cells have suppressed mitochondrial function.

Normally, cells metabolize glucose to pyruvate in the cytoplasm by glycolysis; then, this is converted by pyruvate dehydrogenase (PDH) into acetyl-CoA, which enters the mitochondrial Krebs' cycle, where it is oxidized to eventually produce the electron donors NADH and $FADH_2$. These donate electrons down a redox gradient in the electron transport chain (ETC), while protons are pumped out of the mitochondria and mitochondria-derived reactive oxygen species (mROS; mostly the negatively charged superoxide) are generated, creating a membrane potential ($\Delta\Psi m$) across the mitochondrial inner membrane (Fig. 1). This process uses oxygen as the final electron acceptor in Complex IV of the ETC (forming water) and uses the stored energy of the $\Delta\Psi m$ to produce and release ATP. As protons re-enter the inner membrane, they release energy used to pump ATP out in the cytoplasm and bring ADP in. A similar process is followed in the oxidation of fatty acids, forming acetyl-CoA, which is also oxidized in the Krebs' cycle producing the same electron donors. Thus, mitochondria process fuel (carbohydrates, lipids, oxygen) to produce energy and have evolved to be important fuel sensors. When fuel supply is ample, the growth and differentiation of tissues can be kept under control by the coordinated induction of apoptosis, producing effective cell population control. In contrast, when fuel is limited, mitochondria suppress apoptosis in an attempt to preserve life under stressed conditions. It is here that mitochondria can be "fooled" by the cancer cells.

Let us say, for example, that oxygen is limited. This de facto inhibits oxidative phosphorylation in the mitochondria. Immediately, the mitochondria sense the lack of fuel and ignite a series of mechanisms for the cell to (a) seek alternate means of ATP production and (b) suppress apoptosis since stress may be imminent:

(a) The expression of glucose transporters is increased and more glucose enters the cell. This can be achieved by the activation of HIF1α directly by hypoxia but—remarkably—the mitochondrial suppression can also activate HIF1α. This is because alpha-ketoglutarate (αKG), a Krebs' product that is a required cofactor for the prolyl-hydroxylases that de-stabilize HIF1α, is decreased [7]. Independent of its stabilization, the HIF1α transcription machinery, in part driven by the redox-sensitive p53, may also be activated when the diffusible mROS decrease (for example, H_2O_2 from superoxide's dismutation from manganese superoxide dismutase) [8–11]. Activated HIF1α not only increases the expression of glucose transporters but also increases the transcription of almost all the glycolytic enzymes in the cytoplasm. Thus, with more glucose and activated glycolysis, ATP can be produced. Normally, the efficiency of glycolysis for ATP is less than that of mitochondria (each mole of glucose produces 36 mol of ATP in mitochondrial glucose oxidation but only 2 mol of ATP in glycolysis), but as glycolysis is enhanced, the cell may eventually compensate for the ATP that is missing from the inhibited mitochondria.

(b) The next thing that happens is that mitochondria hyperpolarize (increased $\Delta\Psi m$) [12, 13]. The pro-apoptotic factors stored inside mitochondria (like cytochrome c or apoptosis-inducing factor) are unable to leak out, making cancer cells resistant to mitochondria-dependent apoptosis. This is because these factors leak through the mitochondrial transition pore
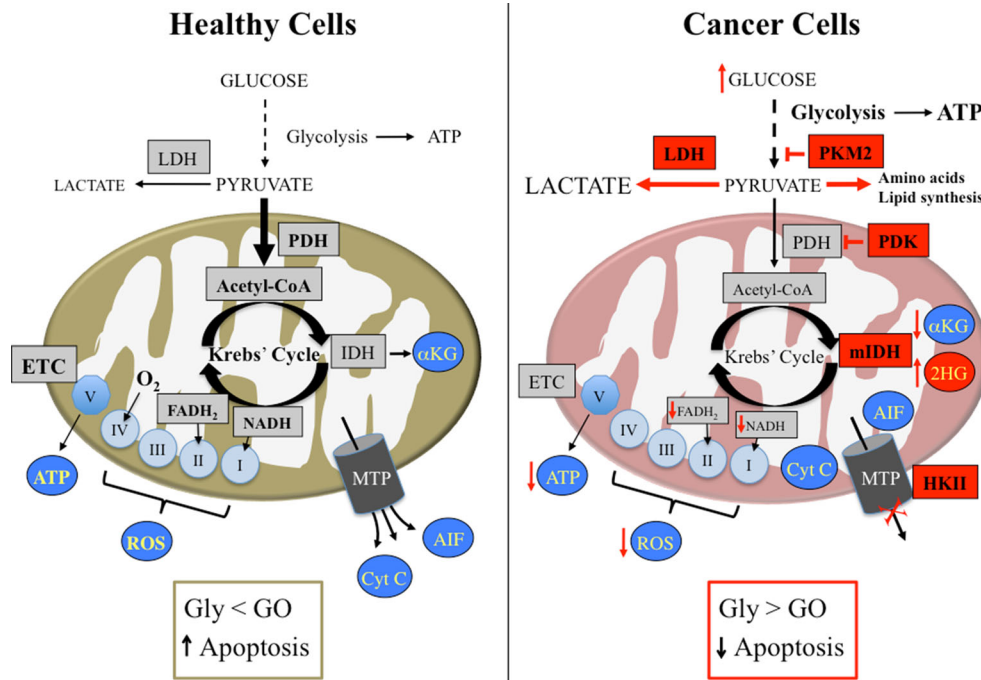
Fig. 1 Suppressed mitochondrial function in cancer cells under normoxia (Warburg Effect). While the mitochondrial glucose oxidation (GO) is suppressed, the cytoplasmic glycolysis (Gly) is enhanced in cancer. This is caused by a combination of inhibited mitochondrial enzymes, whether by upregulation of PDK (a PDH inhibitor), LDH5 and PKM2 (isozymes that confer altered enzymatic activity) or by mutations in IDH. All of these enzymes are induced by HIF1α. On the other hand, the effects of this remodeling include decreased production of αKG (as a result of inhibited Krebs' cycle) and increased levels of 2-HG, the product of mutated IDH. Both of which result in HIF1α stabilization, closing a powerful feedback loop. In addition, this remodeling results in mitochondrial hyperpolarization (in part of a translocation of the glycolytic enzyme HKII to the VDAC, inhibiting the function of the mitochondrial transition pore, a mega channel that allows the release of pro-apoptotic factors like cytochrome c and apoptosis inducing factor, thus inhibiting mitochondria-dependent apoptosis. Pyruvate dehydrogenase kinase (*PDK*), pyruvate dehydrogenase (*PDH*), lactate dehydrogenase 5 (*LDH5*), pyruvate kinase M2 (*PKM2*), isocitrate dehydrogenase (*IDH*), hypoxia inducible factor 1α (*HIF1α*), alpha-ketoglutarate (*αKG*), 2-hydroxyglutarate (*2-HG*), hexokinase II (*HKII*), and voltage-dependent anion channel (*VDAC*)

(MTP), a mega-channel that, being voltage- and redox-sensitive, tends to close while membrane potential is high and mROS are low [14]. The mechanism for mitochondrial hyperpolarization is not clear although it has been known since the 1980s to be a feature of most cancers [15]. One potential mechanism is that glycolysis leads to a GSK-3β-driven translocation of the cytoplasmic hexokinase II (HK2) to the outer mitochondrial membrane, where it binds and inhibits the major channel that releases negatively charged ions, i.e., the voltage-dependent anion channel (VDAC; a critical component of the MTP), leading to a buildup of anions inside the mitochondria [16, 17] (Fig. 1). Another mechanism may be that the enhanced production of ATP in the cytoplasm due to the enhanced glycolysis causes a decrease in the ATP/ADP gradient in the microdomains around the outer mitochondrial membrane, decreasing the function of ATP synthase and thus preventing the re-uptake of the positively charged H$^+$ ions back to the mitochondria [16]. Therefore, mitochondrial suppression supports a state of apoptosis resistance, while adequate production of ATP is maintained. Interestingly, HIF1α also induces pyruvate dehydrogenase kinase (PDK), inhibiting PDH, and thus decreasing the production of acetyl-CoA entering the Krebs' cycle.

Let us now see what would happen if PDH were to be inhibited by other means, not by the hypoxia-mediated activation of HIF1α. Immediately, the mitochondria will sense a lack of fuel entering them since the supply of acetyl-CoA will decrease (despite the fact that the supply of glucose and oxygen to the cell remains normal). They will then ignite the same process described above, leading to resistance to apoptosis. One can follow the same logic and realize that inhibition of any critical enzyme used in glucose or fatty acid oxidation may have the same consequences, "fooling the mitochondria" and inhibiting oxidative phosphorylation, as if there was lack of fuel. This mechanism can be used by cancer cells to inhibit apoptosis, a sine qua non of cancer. At the same time, there are three additional advantages that the cancer cells gain by this "inappropriate" mitochondrial suppression:

First, the cancer cell can now use pyruvate that is not oxidized in the Krebs' cycle, for biomass generation, as the tumor grows [18]. In other words, the unused pyruvate helps a dividing cell create the amino acids, nucleotides, and lipids needed to replicate. Specifically, unused pyruvate can be transaminated to produce amino acids. Similarly, unused pyruvate can be metabolized and shunted into the pentose

phosphate pathway to produce both nucleotides and NADPH, which is required to synthesize lipids [18].

Second, as in the case of decreased production of αKG which contributes to HIF1α activation, there are other downstream signals from mitochondria, like decreased mROS, dysregulation of cytoplasmic calcium, or induction of chaperones that via the retrograde pathway (a series of mechanisms that mitochondria induce under stress to send signals to the nucleus) directly or indirectly activate other pro-proliferative master transcription factors (like NFAT for example) [12, 19–21]. Like HIF1α, NFAT can suppress several aspects of mitochondrial function, enhancing the proliferative potential of cancer cells and driving positively reinforcing feedback loops (proliferative signals→mitochondrial suppression→proliferative signals).

Third, this mitochondrial remodeling promotes angiogenesis, not surprising since this mitochondrial remodeling "mimics" hypoxia, a well-known driver of angiogenesis. While hypoxia and HIF1α activation induce mitochondrial suppression [22], the reverse is also true [10], establishing a powerful feedback loop that sustains angiogenesis even in the absence of hypoxia. The majority of solid tumors experience hypoxia during early tumor development as it occurs several cell layers away from capillaries [23]. This leads to an initial primary hypoxia-driven activation of HIF1α and angiogenic signaling to form new blood vessels to supply the growing tumor with nutrients. However, HIF1α stabilization is also regulated by several diffusible mitochondria-derived factors, like αKG and mROS as discussed above [23]. These lead to the inhibition of the degradation of HIF1α through VHL-dependent ubiquitination or activation of its transcriptional activity, respectively [24, 25]. Thus, as the newly formed blood vessels bring in more oxygen, limiting hypoxia, the mitochondrial suppression that takes place in the tumor now causes a primary normoxic activation of HIF1α, sustaining angiogenesis as the tumor continues to grow [10, 22]. Furthermore, by reducing pyruvate into lactic acid during glycolysis, cancer cells increase extracellular acidosis. This potentiates breakdown of the extracellular matrix and allows penetration of the cancer through the basement membrane, thereby driving metastasis [26].

Thus, mitochondrial suppression promotes suppressed apoptosis, increased proliferation, angiogenesis, and metastatic potential and so can be seen as a critical hub of cancer signaling. While hypoxia is a physiologic mechanism for global mitochondrial suppression, we will now discuss a variety of prominent cancer mechanisms that all lead to mitochondrial suppression, in a sense, mimicking hypoxia.

The cause of mitochondrial suppression in cancer

Mitochondria are well-known "integrators" of multiple signals [12]. Thus, mitochondrial suppression can occur through four overarching mechanisms in cancer: (1) growth and transcription factor signaling or (2) off-target effects of specific oncogenes that can suppress specific enzymes or ETC complexes, (3) suppression of factors regulating mitochondrial homeostasis, and (4) mutations in key metabolic enzymes. It is important to note that more than one of these factors may be present within any given tumor.

1. *Growth Factors*: One of the pleiotropic effects of growth factors upregulated in cancer, such as epidermal growth factor (EGF) and fibroblast growth factor (FGF), is the regulation of the flux of pyruvate into the mitochondria by the gate-keeping enzyme PDH. EGF and FGF increase the activity of pyruvate dehydrogenase kinase (PDK) [27] which phosphorylates and inhibits PDH, thus preventing the entry of pyruvate into the Krebs' cycle and suppressing glucose oxidation (GO) [28]. Furthermore, EGF signaling has recently been shown to also directly inhibit PDH function, through a PDK-independent tyrosine phosphorylation of its E1 subunit [29]. Furthermore, PDK is a target gene of HIF1α, which is upregulated in most solid tumors [23, 30]. HIF1α works at multiple levels to shift the balance of metabolism from GO toward glycolysis, including upregulation of glucose transporters and glycolytic enzymes. HIF1α also increases the translation of the EGF receptor, thereby suppressing mitochondrial function by both upregulating and activating PDK [31]. There are four PDK isoenzymes with variable tissue expression, and some, like PDK 4, are inducible in conditions of metabolic stress [32]. Thus, it is reasonable to assume that other, not yet identified, "fuel sensing" mechanisms exist in inducing the transcription of one or more PDKs in tumors, in addition to increasing PDK activity through tyrosine kinase signaling.

2. *Oncogenes*: Mitochondrial function is also suppressed by many oncogenes, with p53 and c-MYC being prime examples. The Cancer Genome Atlas has identified p53 to be the most commonly mutated gene in cancer [33]. p53 inhibits glycolysis by inducing the expression of Tp53-induced glycolysis and apoptosis regulator (TIGAR) as well as by reducing the expression of glycolytic enzymes, like phosphoglycerate mutase [34, 35]. Furthermore, p53 enhances GO by increasing the expression of cytochrome c oxidase subunits of the ETC [36]. Loss of p53 function causes upregulation of both the glycolytic enzyme hexokinase II (HKII) and PDK [37, 38]. Overall, loss of p53 function suppresses mitochondria and shifts the cell to a more glycolytic phenotype. It is important to note that post-translational modifications like acetylation, a process intimately linked to mitochondrial function, are also known to regulate p53's function in cancer and in the absence of mutations [39]. Like p53, c-MYC increases the expression of multiple glycolytic enzymes including

HKII, phosphofructokinase, GAPDH, and enolase A, as well as glucose transporters and lactate dehydrogenase (LDH) [40–42]. On top of driving glycolysis, upregulation of LDH shifts the flux of pyruvate away from GO, thereby further suppressing mitochondrial function.

3. *Regulation of factors that control mitochondrial homeostasis*: Global mitochondrial function can be regulated through inhibition of mitochondria-specific factors like sirtuin 3 (Sirt3; the main de-acetylase in the mitochondria) and uncoupling protein-2 (UCP-2; a putative mitochondria calcium transporter). Inhibition of Sirt3 and UCP-2 suppresses mitochondrial function by increasing protein acetylation and decreasing mitochondrial calcium, respectively. When Sirt3 is deficient, the acetylation of the majority of proteins involved in oxidative metabolism, including Krebs' enzymes and ETC complexes, increases; this post-translational modification typically leads to the inhibition of enzymatic function [43, 44]. Overall, loss of Sirt3 activity can cause up to 50 % reduction in mitochondria-derived ATP and respiration. Thus, it is not surprising that loss of Sirt3 function has been shown to promote the Warburg Effect and cancer. In fact, embryonic fibroblasts lacking Sirt3 require activation of only one other oncogene to transform into cancer [45], in contrast to wild-type cells that require at least two. Moreover, mice deficient in Sirt3 spontaneously develop breast cancer, and many human cancers are deficient in Sirt3 [45–47]. These observations have led investigators to propose that Sirt3 fulfills the criteria to be an oncogene [45].

Similarly, loss of UCP-2 function, which despite its name is a weak uncoupler but good mediator of calcium entry into the mitochondria, promotes proliferation [20] in part due to a reduction in mitochondrial calcium, which decreases activity of many calcium-dependent mitochondrial enzymes such as PDH, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase [48, 49]. Mice deficient in UCP-2 are more prone to develop colon cancer than wild-type UCP-2 littermates when exposed to a carcinogen, demonstrating a predisposition to oncogenic transformation in these glycolytic animals [50]. Similarly to Sirt3, UCP2 was recently proposed to be a tumor-suppressing factor [20].

Another way that mitochondrial function can be suppressed globally is disturbance of the way mitochondria form networks in the cell (mitochondrial fission and fusion) or the way these organelles are "recycled" (mitophagy) [51]. Since these mitochondrial properties are closely linked to cell cycle progression and cell division, it is not surprising that there is early evidence that they may be involved in carcinogenesis [52, 53]. Yet, the importance of these essential functions for many dividing healthy cells may limit their therapeutic potential.

4. *Enzymatic mutations*: Mutations in Krebs' cycle enzymes lead to suppressed mitochondrial function. Three examples of this are fumarase, succinate dehydrogenase (SDH), and isocitrate dehydrogenase (IDH). Mutations in fumarase have been identified in almost all cases of the tumor susceptibility syndrome, hereditary leiomyomatosis, and renal cell carcinoma [54]. Similarly, SDH mutations have been linked to the development of pheochromocytoma, paraganglioma, and renal cell carcinoma [55, 56]. Loss of fumarase or SDH function leads to an accumulation of intracellular fumarate or succinate, respectively. Each of these Krebs' metabolites has been shown to stabilize HIF1α by inhibiting the prolyl-hydroxylases required for HIF1α degradation [57–59]. This leads to a high level of HIF1α activity even in the presence of oxygen. As a result, these tumors are very vascular. Similarly, a powerful pseudohypoxic environment is created in tumors housing IDH mutations due to gain-of-function activity. Two independent cancer genome sequencing projects found that missense mutations in patients with glioblastoma multiforme and acute myelogenous leukemia caused substitutions of a homologous arginine in the active site of the enzyme at R132 and R172 in IDH1 and IDH2, respectively [60, 61]. These mutations cause a gain-of-function, neomorphic activity, in which the mutant IDH-mediated reaction yields 2-hydroxyglutarate (2-HG) rather than the normal product, αKG [62, 63]. Nearly structurally identical to αKG, 2-HG may competitively inhibit the over 60 αKG-dependent dioxygenase enzymes in humans by binding to the αKG binding pocket in the enzyme's active site [7]. These αKG-dependent enzymes (and thus 2-HG targets) are involved in a broad range of biological processes including HIF1α degradation, collagen synthesis, and histone methylation [64]. 2-HG also alters HIF1α degradation by inhibiting HIF prolyl-hydroxylases. As a result, cells producing 2-HG, due to an IDH mutation, are locked into a state of pseudohypoxia and mitochondrial suppression. Similar to renal cell carcinoma, glioblastomas are also particularly vascular tumors.

Like p53, many of the Krebs' enzymes, including those discussed above, can be (at least partially) inhibited via post-translational modifications like acetylation, even in the absence of mutations, and this has also been shown to be present in several cancers [65, 66].

Mitochondria-targeting cancer therapies

We will now follow the sequential metabolism of glucose in cancer cells to highlight several cancer-specific metabolic targets that have been explored (Fig. 2), focusing on the translational potential of these discoveries.

Glucose transport and phosphorylation Since glycolysis is upregulated in many cancers, it may appear logical to attempt to inhibit it at its early stage. Glycolysis starts by glucose entry into the cell, through glucose transporters (GLUTs), followed immediately by phosphorylation by hexokinase (HK), required to "trap" glucose intracellularly. Logically, pharmacological inhibition of these two proteins may make sense as both are upregulated in many cancers in order to increase glucose uptake and glycolysis and compensate for the loss of GO. However, being so proximal in the metabolic pathway and ubiquitous in all cells, inhibition of GLUTs and HK, while effective in some pre-clinical studies, has suffered setbacks when tested in early phase human trials [67, 68]. For example, inhibition of GLUTs led to brain toxicity as neurons rely mostly on the use of carbohydrate metabolism [69]. Similarly, HK inhibition led to severe hepatic toxicity, an organ heavily involved in both catabolism and anabolism of glucose [69]. In fact, more than half of the clinical trials using the GLUT and HK inhibitors, 2-deoxyglucose and lonidamine, were terminated prematurely [70]. Nevertheless, the HK inhibitor, 3-bromopyruvate, which showed good effect in a xenotransplant model, was used in a patient with fibrolamellar hepatocellular carcinoma [71, 72]. While this patient survived the duration of therapy, with few reported serious systemic side effects, the drug was delivered directly to the tumor-related artery by transarterial chemoembolization (TACE) [73], perhaps limiting toxicity. Inhibiting glycolysis follows a more traditional "cytotoxic" pathway as inhibition of glycolysis causes non-specific necrosis; in fact, suppressing glycolysis will unavoidably further suppress mitochondrial function as it deprives mitochondria from a primary fuel in most tissues, limiting the therapeutic potential of this strategy. This is in contrast to the metabolic modulators discussed below that target the "coupling" of glycolysis to GO, actually enhancing mitochondrial function, allowing mitochondria to operate their intrinsic apoptotic machinery (an energy consuming function) or normalize their downstream signaling. While it is easy to criticize retrospectively, the investigators of these early clinical studies should be given credit as the first that attempted to target a metabolic process in human cancer, contributing to our re-examination of Warburg's "forgotten" theory.

Pyruvate kinase M2 (PKM2) activation Pyruvate stands at a crossroads of metabolic fates: It is the product of cytoplasmic glycolysis, the product of cytosolic malate oxidation (to make anabolic NADPH), a precursor for amino acid production through transamination, the substrate of PDH versus LDH to either make acetyl-CoA which drives the mitochondrial Krebs' cycle, or lactate and complete glycolysis, respectively [69, 74, 75]. Pyruvate kinase (PK) is the last enzymatic step in glycolysis, catalyzing the reaction of phosphoenolpyruvate to pyruvate, and consists of four isoforms [76]. Two of these isoforms, M1 (PKM1) and M2 (PKM2), are encoded by alternative splicing of the PKM2 (15q23) gene [77, 78]. Enzymatically, PKM1 is the highly active version and is found
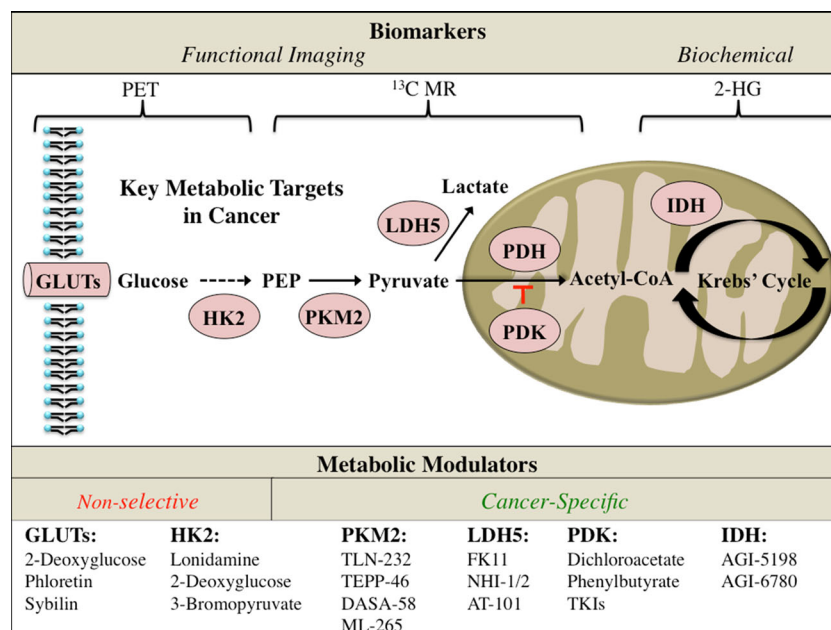

Fig. 2 Biomarkers and metabolic modulators arising from the metabolic theory of cancer (see text). Biomarkers (*top*) and metabolic modulators (*bottom*) that have been developed for target enzymes and have been or are in preclinical or clinical trials. Glucose transporter 1 (GLUT1), Hexokinase II (HKII), M2 isoform pyruvate kinase (PKM2), Lactate dehydrogenase 5 (LDH5), Pyruvate dehydrogenase (PDH), pyruvate dehydrogenase kinase (PDK), Tyrosine Kinase Inhibitors (TKIs) Isocitrate dehydrogenase (IDH), Phosphoenolpyruvate (PEP), Positron emission tomography (PET), Magnetic resonance (MR), 2-hydroxyglutarate (2-HG)

in normal tissues requiring large amounts of glucose-derived ATP, like skeletal muscle or brain [79, 80]. In contrast, the less active PKM2 is expressed in most tissues during development and has been found in many cancer cell lines [80–82]. Indeed, preferential expression of PKM2 over PKM1 is associated with mitochondrial suppression and enhanced tumorigenesis [80, 83, 84]. The enzymatic activity of PKM2 is regulated by several factors including glycolytic intermediates, tyrosine phosphorylation, and acetylation [85–87]. Therefore, even though PKM2 expression is increased in cancer, its overall activity may be decreased [80]. Furthermore, it is dynamic since in nutrient-abundant states, PKM2 forms active tetramers that function similarly to the more active PKM1 [80, 88]. The importance of this dynamic regulation of PKM2 has recently been explored in cancer [89]. Israelson et al. found that mice conditionally deficient in PKM2 had a more accelerated tumor growth and mortality than their wild-type littermates. Furthermore, the ratio of the inactive over active form of PKM2 was found to tip the balance toward cancer growth. In contrast to the earlier belief that it was the switch from one isozyme to the other that promotes cancer, this work showed

that it is the overall suppression of PKM1/2 activity as a whole (and thus the suppressed GO) that promotes cancer (Fig. 3a). Indeed, overexpressing PKM1 in cancer cell lines lead to reduced tumor growth in xenotransplant models [83]. Thus, while the early discovery of an isozyme "specific for cancer" would have triggered efforts to inhibit it, it appears that it is PKM2 activators that may hold promise as cancer therapies. Several small molecules have been developed, such as TEPP-46, DASA-58, and ML265, which bind PKM2 at the subunit interaction interface to promote formation of enzymatically active tetramers [90]. This leads to a constitutively active enzyme with over 200 % enhanced activity [83]. In vitro, treatment with these small-molecule activators decreased the intermediates necessary for biomass generation, reduced lactate production and lipid synthesis, and lead to smaller and slower growing tumors in vivo [83, 91].

Despite these promising results, pharmacologic activation of PKM2 may not induce cytotoxic changes in cancer. While populations of cancer cells expressing PKM2 do not proliferate, they continue to persist in a non-proliferative, perhaps senescent state [89]. Therefore, once a tumor has been
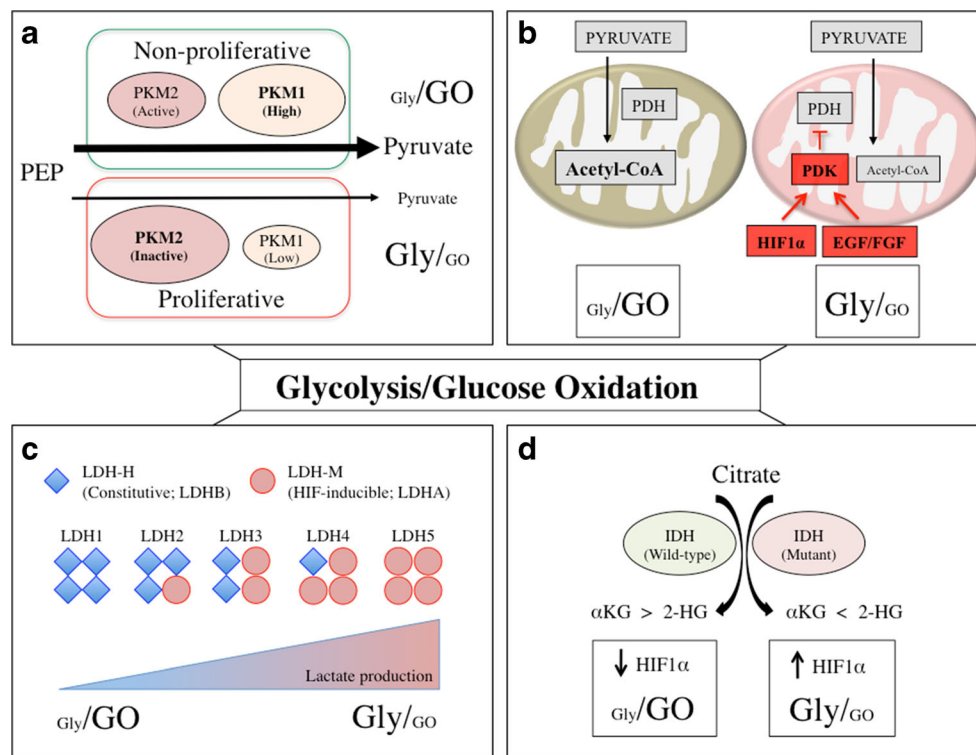

Fig. 3 Ratio of Glycolysis to Glucose Oxidation increases in cancer due to changes in several key metabolic enzymes. (a) Cells that express a low amount of Pyruvate kinase M1 (PKM1) and an abundance of low-activity Pyruvate kinase M2 (PKM2; which results in an overall decrease in pyruvate kinase activity) prevent the entry of pyruvate into the mitochondria, inhibiting glucose oxidation (GO). (b) Pyruvate dehydrogenase kinase (PDK) also inhibits the entry of pyruvate into the mitochondria by phosphorylating and inhibiting pyruvate dehydrogenase (PDH). (c) The Lactate dehydrogenase (LDH) enzyme is made from four subunits comprised of H (Heart) or M (Muscle) isoforms. Overexpression of the hypoxia-inducible factor 1α (HIF1α) inducible LDH-M creates an enzyme comprised of four M subunits resulting in increased activity, favouring the reduction of pyruvate to lactate, thereby shunting pyruvate away from GO. (d) Mutation in isocitrate dehydrogenase (IDH) leads to the production of the oncometabolite, 2-hydroxyglutarate (2-HG), which antagonizes the normal product, alpha-ketoglutarate (αKG), leading to suppressed GO via the stabilization and accumulation HIF1α

detected, treatment with PKM2 activators may only limit further growth and would need to be combined with another agent or treatment modality to debulk the tumor by increasing cytotoxicity or by inducing apoptosis.

Pyruvate dehydrogenase kinase (PDK) inhibition PDK phosphorylates and inhibits the E1a subunit of PDH [28]. The net result of inhibited PDH is an increase in the ratio of glycolysis over GO (Fig. 3b), with all the subsequent downstream signaling events that were discussed earlier. It is possible that increased expression and activity of PDK (via HIF1α or tyrosine kinase signaling as discussed earlier) is enough to induce the Warburg Effect and be the dominant mechanism in certain cancers [13, 92] or other proliferative diseases, like pulmonary arterial hypertension, characterized by a proliferative vascular remodeling and mitochondrial suppression [12, 93]. For example, PDK is significantly more increased in glioblastoma tumors compared to that in healthy brain tissues from the same patient [94], although this has not been systematically studied in cancer yet. There is strong evidence that PDK inhibition decreases cancer growth in vitro and in vivo in a variety of tumors as discussed below.

Dichloroacetate is an orally available small-molecule inhibitor of PDK (structurally resembling pyruvate) that can reach most tissues and cross the blood brain barrier. Dichloroacetate (DCA) inhibits PDK at concentrations of 10–250 μM, while it is more active against some of the four PDK isoforms (i.e., the ubiquitously expressed PDK2) compared to others [95, 96]. DCA's mechanism of action is quite specific as it is mimicked by molecular PDK knockdown; in addition, DCA has no additional effects in cells with effective PDK knockdown [94, 97].

Originally, DCA was pioneered by Dr. Stacpoole's group at the University of Florida to limit lactic acidosis in children with congenital mitochondrial diseases (for example, deficiencies of PDH or other mitochondrial enzymes), and over the past 40 years, it has been explored in a number of disease states associated with lactic acidosis or with a primary mitochondrial suppression, including diabetes, malaria, pulmonary arterial hypertension, lactic acidosis, heart failure, and exercise tolerance in chronic respiratory disease [98–106]. In 2007, we described DCA's pro-apoptotic and antiproliferative effects due to normalization of mitochondrial function in a variety of cancers (non-small-cell lung cancer, breast cancer, glioblastoma) in vitro and in xenotransplant models in vivo (Fig. 4a) [97]. DCA, a generic drug, cannot be patented, creating the potential for financial barriers in its development as a cancer treatment. Yet, a number of investigators have shown interest since and have described similar effects in a variety of tumors. Some examples include prostate cancer [107], colon cancer [108–110], gastric cancer [111], endometrial cancer [112], glioblastoma [113], neuroblastoma [114], T cell lymphoma [115], non-Hodgin's lymphoma [116], fibrosarcoma [117], and metastatic breast cancer [118] (Table 1). In this last study, DCA reduced lung metastases by 58 % in a highly metastatic breast cancer model [118].

DCA appears to not have significant effects in normal cells, perhaps because of low levels of PDK activity in healthy tissues. Generally speaking, normal cells need active mitochondria with active PDH and keep the levels of its inhibitor (PDK) low. For example, DCA normalized the high $\Delta\Psi$m of non-small-cell lung cancer, glioblastoma, and breast cancer cell lines without altering the $\Delta\Psi$m of each cancer's non-malignant tissue analog, i.e., small airway epithelial cells, mammary epithelial cells, or healthy brain tissues [10, 94, 97].

Fig. 4 Translation of DCA from animal studies to an early phase human trial. DCA decreased tumor size (**a**), vascularity and FDG uptake (measured by microPET-CT (**b**) in a xenograft rat model with non-small cell lung cancer. In a small human glioblastoma trial, DCA normalized mitochondrial metabolism, increased apoptosis, suppressed angiogenesis and reduced tumor growth after debulking surgery for at least 18 months, in a patient that had failed all approved therapies and was otherwise destined for hospice care (**c**)
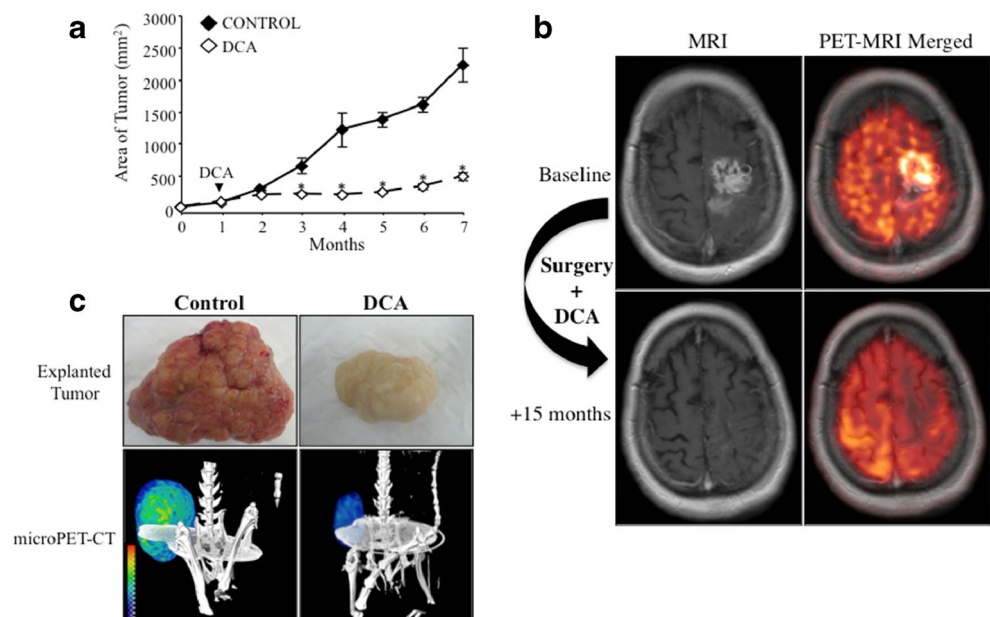
Table 1 Early evidence for dichloroacetate's potential benefits in multiple cancer types

Cancer type	Model	Outcome	Monotherapy vs Combination	Reference
Breast	In vivo lung metastasis model	Reduced number of lung metastases, increased apoptosis	Monotherapy	[118]
Breast	Xenotransplant	Reduced tumor growth and angiogenesis	Monotherapy	[10]
Colon	Xenotransplant	Reduced tumor growth and restored mitochondrial ultrastructure	Monotherapy	[108]
Colon	In vitro	Reduced proliferation and increased apoptosis	Monotherapy	[109]
Colon	Spontaneous colorectal adenocarcinoma	Decreased tumor grade, tumor size, and number of tumors formed	Monotherapy	[110]
Endometrial	In vitro	Increased apoptosis	Monotherapy	[112]
Fibrosarcoma	In vitro	Increased apoptosis	Combination with Tamoxifen and Omeprazole	[117]
Gastric	In vitro	Increased apoptosis and sensitized resistant cancer cells to 5-fluorouracil	Combination with 5-fluorouracil	[111]
Glioblastoma	In vitro	Increased apoptosis	Monotherapy	[97]
Glioblastoma	Xenotransplant	Reduced tumor growth	Combination with bevacizumab	[113]
Neuroblastoma	Xenotransplant	Reduced tumor growth	Monotherapy	[114]
Non-Hodgkin's Lymphoma	In vivo	Complete remission on PET	Monotherapy	[116]
Non-small cell lung	Xenotransplant	Reduced tumor growth and increased apoptosis in vivo	Monotherapy	[97]
Prostate	In vitro	Increased apoptosis	Combination with external beam radiation	[107]
T-cell lymphoma	In vitro	Increased apoptosis Reduced HIF1α function	Monotherapy	[115]

In addition to the induction of apoptosis and inhibition of proliferation, DCA can exhibit effects apparent only in vivo, as it suppresses angiogenesis by reversing the pseudohypoxic state caused by activated PDK. By promoting the decarboxylation of pyruvate into acetyl-CoA, DCA drives the Krebs' cycle to produce αKG, as well as NADH and $FADH_2$ to be used in the ETC [10]. This leads to increased mROS, which increases activity of redox sensitive tumor suppressors like p53 [10, 13, 94, 115]. Together, increased p53 activity and increased levels of αKG prevent the stabilization of HIF1α as well as reduce HIF1α transcriptional activity and the expression of downstream HIF1α targets [10, 119]. DCA reduces the levels of angiogenic signaling molecules such as VEGF and SDF-1 and prevents neovascularization both in vitro and in xenotransplant tumor models [10]. In addition, inhibition of PDK using RNA interference has recently been shown to promote oncogene-induced senescence in melanoma in vitro and in vivo, providing another mechanism through which DCA may be exerting antitumor effects [120].

DCA has had success in early-phase small clinical trials for glioblastoma (GBM) and recurrent brain tumors [94, 121]. Compared to healthy brain tissue removed during surgery for epilepsy, tumors from 49 patients with GBM exhibited significantly higher levels of PDK and hyperpolarized ΔΨm. Treatment of five patients with DCA (for which brain tissue was removed at the time of debulking surgery at baseline as well as after DCA treatment, allowing direct pre-post comparisons)

caused mitochondrial depolarization, increased rates of apoptosis, activated p53, reduced proliferation, and inhibited HIF1α activity and tumor vascularity [94]. Despite the very advanced stage of their disease, some patients showed evidence of tumor regression whereas others remained clinically stable for >18 months (Fig. 4b). No patient developed hematologic, hepatic, renal, or cardiac toxicity. Peripheral neuropathy developed in some patients but reversed at a lower dose of DCA. Similar results, supporting the safety of the drug and the need for phase II trials in glioblastoma, were confirmed by another phase I trial performed by an independent group, demonstrating clinically stable disease with no significant side effects beyond peripheral neuropathy, which when it occurs is dose-dependent and reversible [121]. Recently, the University of Florida group published their experience with DCA in children that were treated with DCA in their group continuously from 9.7 to 16.5 years at a dose of 12.5 mg/kg two times a day (i.e., higher or equal to the doses used in the glioblastoma trials in humans). They reported no hematological, electrolyte, renal, or hepatic toxicity, with the only toxicity being a reversible and dose-dependent peripheral neuropathy (treated with dose reduction or only temporary discontinuation of DCA) [122].

Although the initial half-life of DCA is very short (i.e., approximately 2 h) [96], the drug inhibits its own metabolism until it reaches a plateau, and thus therapeutic concentrations can be achieved in plasma with time (for example, at a dosing

regimen of 6.25 mg/kg two times a day for 3 months) [94]. Nevertheless, this may take more than 3 months. Thus, DCA may not be a good choice as a monotherapy in advanced and rapidly proliferating tumors. Like the PKM2 targeting drugs, it is not cytotoxic and could be seen as a drug that "sensitizes" the tumors to apoptosis, perhaps best used as a part of a combination therapy with a more cytotoxic drug at the early stages of treatment.

Another fact that may support DCA's sustained effects in the long term is its ability to inhibit HIF1α. For example, the early effectiveness of VEGF inhibitors is limited by the eventual escape of the tumor, which, having sustained HIF1α activity, can continue to generate alternate pro-angiogenic factors. Thus, metabolic modulators like DCA that have the ability to inhibit the normoxic activation of HIF1α may offer much more sustained and effective inhibition of angiogenesis [10]. Indeed, synergy between DCA and VEGF inhibitors was recently shown in glioblastoma cancer models [113].

The anticancer efficacy of DCA increases when combined with other agents. Similar to the synergy between DCA and VEGF inhibitors or DCA and temozolomide, a combination agent, called mitaplatin, combines DCA with cisplatin [123]. This unique drug is synthesized by adding two DCA moieties (one onto each end) of a cisplatin core. Once this drug enters a cancer cell, it is reduced to release one molecule of free cisplatin and two molecules of free DCA. The result is the combined effect of DCA on mitochondria (increasing cytochrome c release and apoptosis) as well as cisplatin-mediated DNA crosslinking. Mitaplatin exceeded the anticancer efficacy of cisplatin alone in a variety of cancer cell lines [123]. DCA has also been used in combination with 5-fluorouracil (5-FU) to re-sensitize hypoxic gastric cancer cells that developed resistance to 5-FU monotherapy [111]. Similarly, the combination of DCA with tamoxifen and omeprazole exhibited more potent antitumor activity than those agents alone [117]. Furthermore, other therapeutic modalities, such as external beam radiation, have been found to be efficacious in combination with metabolic modulators. DCA sensitized prostate cancer cells (which were previously resistant due to overexpression of BCL-2), to radiation therapy [107]. Furthermore, in combination with etoposide or irradiation, DCA decreases the apoptosis resistance seen in gliomas compared to treatment with either of these agents alone [124]. These examples suggest that mitochondrial activation may be effective in combination strategies for several tumor types.

DCA's proven ability to increase the GO/glycolysis ratio in the treated tumors and its ability to decrease HIF1α activity and thus reverse the upregulation of glucose transporters suggest that metabolic imaging, like FDG-PET, may be used to track its effects in vivo, a very desirable tool in drug development (Fig. 4c). Tumor cells, expressing a relative abundance of glucose transporters and glycolytic enzymes take up much more fluorinated deoxyglucose (a metabolite that once uptaken

remains trapped intra-cellularly allowing its imaging) than surrounding non-cancerous tissue. In theory, one of the first signs of DCA's effectiveness in vivo maybe its ability to decrease the FDG uptake under FDG-PET imaging, a possibility that although has been documented in animal models [10], should be systematically pursued in future clinical trials.

Lactate dehydrogenase A (LDHA) inhibition Suppressed mitochondria in cancer cells force pyruvate to be reduced to lactate in order to allow glycolysis to continue. This is achieved by lactate dehydrogenase (LDH), a tetrameric enzyme that facilitates the recycling of NAD+ from NADH by reducing pyruvate to lactate in the cytoplasm. There are five isoforms of LDH made from differing subunit combinations of the products from two genes, LDHB and LDHA: LDHB expresses a constitutively active form, LDH-H (heart); LDHA is a HIF1α responsive gene that transcribes a more efficient enzyme, LDH-M (muscle) [74, 125] (Fig. 3c). In highly glycolytic tumors, the isoform made exclusively from four subunits of LDH-M, known as LDH5, predominates [126]. Similarly, tumors epigenetically silence the LDHB gene through hypermethylation of its promoter region, thereby further shifting the ratio of LDH toward LDH5 [127]. Indeed, the increased expression of this highly active tetramer is a marker of poor prognosis in multiple malignancies [126, 128, 129]. In tissues where LDH activity is enhanced, its inhibition will facilitate pyruvate's entry into the mitochondria (assuming that PDH is active), increasing GO and preventing the shift of pyruvate's metabolism into anabolic precursors.

Indeed, inhibition of LDH5 with short hairpin RNA enhanced respiration and reduced $\Delta\Psi$m. LDHA knockdown reduced cancer growth rates in vitro and in vivo in animal models [130]. This work led to the development of a small-molecule inhibitor of LDH, FX11, which was shown to be effective in animal models of lymphoma and pancreatic cancer [131]. Another class of LDH5 competitive inhibitors, N-hydroxy-2-carboxy-substituted indoles, called NHI-1 and NHI-2, has been developed [132, 133]. These more specific and efficient LDH5 inhibitors decrease lactate production and reduce proliferation in multiple cancer cells lines.

Recently, ^{13}C-labeled magnetic resonance spectroscopy has been adapted to follow dynamic metabolic conversions in vivo [134]. This imaging biomarker assesses real-time changes in intracellular metabolism such as decreased reduction of pyruvate to lactate in response to drugs like LDH inhibitors or DCA [135, 136].

Mutant isocitrate dehydrogenase (IDH) inhibition As discussed above, mutant IDH leads to pseudo-hypoxic signaling, due to the production of 2-HG (Fig. 3d). Recently, pharmacological inhibition of IDH has been explored. Several small-molecule inhibitors that specifically inhibit the mutant form of IDH have been developed [137, 138]. Discovered

through high-throughput screening, AGI-5198 inhibits the production of 2-HG by mutant IDH1 while AGI-6780 inhibits 2-HG production by mutant IDH2. This inhibition appears highly specific for the mutant isoform as AGI-5198 impairs only the growth of IDH1 mutant but not IDH1 wild-type glioma xenotransplant tumors [138].

2-HG-producing tumors cause exponentially higher levels of this metabolite in the patient's circulation [63]. Fathi et al. have taken advantage of this unique cancer metabolite, to non-invasively diagnose and subsequently follow response to treatment in AML by detecting the levels of 2-HG in patients' serum and urine samples before and during therapy [139]. While IDH inhibitors have yet to be tested in humans, this powerful biomarker will facilitate IDH inhibitor development in clinical trials.

Cancer stem cells Mitochondrial metabolism also determines stem cell fate. Temporally, a switch to glycolysis precedes expression of stem cell markers and subsequent entry into a pluripotent state [140]. Conversely, an increase in mitochondrial glucose oxidation is necessary for initiating stem cell differentiation [141]. Normal stem cells, similar to cancer cells, exhibit increased LDHA, HKII, PDK, and phosphorylated PDH, compared to differentiated progeny, leading to mitochondrial suppression [142]. This mitochondrial suppression is further exacerbated in hypoxic tumor cells and cancer stem cells. For example, when lung cancer stem cells (LCSCs) are directly compared to differentiated lung cancer cells, LCSCs demonstrate lower oxygen consumption, mROS, mitochondrial numbers, and ATP levels, as well as higher mitochondrial membrane potential [143]. Similarly, when studied in parallel, healthy brain tissue, glioblastoma cancer cells, and glioblastoma putative cancer stem cells exhibit a graded increase in mitochondrial suppression, with the highest levels of $\Delta\Psi m$ seen in the cancer stem cells [94]. Mechanistically, overwhelming mitochondrial suppression in cancer stem cells provides significant resistance to apoptosis, potentially contributing to the cancer stem cells' resistance to conventional chemotherapeutics.

Activating mitochondria unlocks apoptosis resistance in cancer stem cells. For example, tumor biopsies from GBM patients treated with DCA found induction of apoptosis in glioblastoma putative cancer stem cells, particularly when used in combination with temozolomide in vivo and in vitro [94]. Similarly, DCA reduces cancer stem cell viability in embryonal cancer stem cells when the substrate for PDH, pyruvate, is available [144].

Despite the induction of apoptosis in cancer stem cells, there is evidence that DCA exhibits specificity for cancer stem cells and not healthy stem cells. For example, patients treated with long-term (up to 16 years) DCA did not suffer any hematological side effects, suggesting lack of effects on bone marrow stem cells [122]. Yet, it is possible that more potent

mitochondrial activators may have this problem, an important issue that has to be addressed in the future with long-term studies. On the other hand, metabolic disturbances may affect the ability of stem cells to differentiate and, in the case of IDH mutations in leukemic cells, there is evidence that they may impair the ability of hematopoietic stem cells to differentiate, resulting in leukemias that mimic a difficult to treat, hematopoietic stem cell phenotype [145].

Glucose oxidation and histone acetylation Histone acetylation has received a lot of attention in cancer research [146]. As the source of the acetyl group is acetyl-CoA (a prime mitochondrial product), it is possible that the mitochondria suppression discussed herein may actually also impact epigenetic mechanisms. Isolated nuclei exposed to acetyl-CoA exhibit increased histone acetylation [147]. Intriguingly, the acetyl-CoA molecule is extremely unstable and has to be used in the organelle that is produced. In other words, acetyl-CoA cannot simply leak out of mitochondria and enter the nucleus. Recent work, however, has shown two mechanisms by which mitochondria can regulate histone acetylation:

(a) PDH activity promotes citrate production in the Krebs' cycle, which can diffuse out of the mitochondria and into the nucleus to be used as a substrate to acetylate histones by the enzyme ATP-citrate lyase, which is present both in the cytoplasm and the nucleus [148]. Thus, a primary inhibition of PDH will also result eventually in a suppression of citrate production and histone acetylation, unless
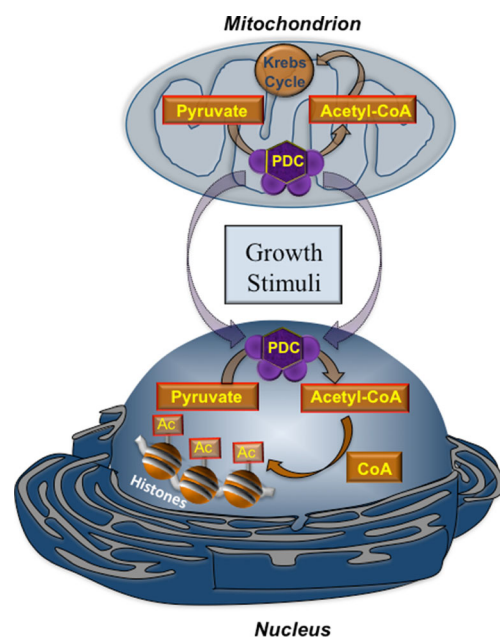

Fig. 5 Nuclear translocation of the PDH Complex (*PDC*) provides Acetyl-CoA for histone acetylation. PDC dynamically translocates from the mitochondria to the nucleus in response to growth factors, like epidermal growth factor (EGF), to provide acetyl-CoA for histone acetylation and cell cycle progression

citrate can be replenished by an alternate pathway like the reductive glutamine pathway, which can produce citrate in the cytoplasm from the amino acid glutamine [149].

(b) We recently described an alternate way of nuclear production of acetyl-CoA by showing that PDH can actually translocate into the nucleus in a cell-cycle-dependent manner [150]. Interestingly, several subunits of the PDH complex, E1a and E2, had previously been shown to be present in the nucleus of leukemic T cells, although at the time their presence was not linked to the main function of PDH, i.e., production of acetyl-CoA [151]. We found that nuclear PDH (which included all subunits of the complex), although in small amounts, is functional, providing a source of acetyl-CoA to be used to acetylate specific histone residues involved in cell cycle progression [150]. Intriguingly, PDK does not follow PDH in the nucleus (potentially being displaced from its binding site on the E2 subunit by HSP70, which then transports a "PDK-free" HSP70-PDH complex to the nucleus), suggesting that nuclear PDH will be immune to DCA and perhaps represent a potential "escape" mechanism to DCA treatment (Fig. 5) [150].

The fact that translocation of such a large enzyme like PDH takes place between mitochondria and the nucleus is not as surprising as one may first think. For example, while mitochondrial PDH has been classically thought to be localized in the mitochondrial matrix, it has also been shown to move through the mitochondrial inner membranes and remain functional in the outer mitochondrial membrane [27]. This is in keeping with our finding that the chaperone HSP70 may be involved in its nuclear translocation since it may easily reach PDH on the outer mitochondrial membrane [150]. The role of nuclear PDH is not entirely clear but suggests that it may provide a critical regulatory mechanism for gene expression by mediating a shift from heterochromatin to euchromatin thereby facilitating transcription factor binding. Identifying a mechanism by which this translocation is blocked may represent a new means of cancer therapy, merging the fields of metabolism and epigenetic regulation therapeutics.

Conclusion

Suppressed mitochondrial function and in particular GO appears to be a universal feature of cancer, giving cancer cells a proliferative advantage, while simultaneously repressing apoptosis. A multitude of mechanisms underlie this mitochondrial remodeling, exposing a number of novel therapeutic targets. Although not cytotoxic, limiting their use as monotherapies in rapidly growing tumors, these metabolic modulators appear to be selective to the tumor. Metabolic oncology also has the potential to utilize a number of unique metabolism-based biomarkers. Multiple small molecules targeting both cytoplasmic and mitochondrial enzymes involved in cancer metabolism, such as PKM2, PDK, IDH, and LDH5, are currently in use in preclinical and early phase human clinical trials, a remarkable achievement of translational oncology that was essentially born less than 10 years ago.

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