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Review

REVIEW

Therapeutic Targeting of the Pyruvate Dehydrogenase Complex/Pyruvate Dehydrogenase Kinase (PDC/PDK) Axis in Cancer

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Abstract

The mitochondrial pyruvate dehydrogenase complex (PDC) irreversibly decarboxylates pyruvate to acetyl coenzyme A, thereby linking glycolysis to the tricarboxylic acid cycle and defining a critical step in cellular bioenergetics. Inhibition of PDC activity by pyruvate dehydrogenase kinase (PDK)–mediated phosphorylation has been associated with the pathobiology of many disorders of metabolic integration, including cancer. Consequently, the PDC/PDK axis has long been a therapeutic target. The most common underlying mechanism accounting for PDC inhibition in these conditions is post-transcriptional upregulation of one or more PDK isoforms, leading to phosphorylation of the E1α subunit of PDC. Such perturbations of the PDC/PDK axis induce a "glycolytic shift," whereby affected cells favor adenosine triphosphate production by glycolysis over mitochondrial oxidative phosphorylation and cellular proliferation over cellular quiescence. Dichloroacetate is the prototypic xenobiotic inhibitor of PDK, thereby maintaining PDC in its unphosphorylated, catalytically active form. However, recent interest in the therapeutic targeting of the PDC/PDK axis for the treatment of cancer has yielded a new generation of small molecule PDK inhibitors. Ongoing investigations of the central role of PDC in cellular energy metabolism and its regulation by pharmacological effectors of PDKs promise to open multiple exciting vistas into the biochemical understanding and treatment of cancer and other diseases.

Energy is life. For animals and most other life forms on this planet, life depends on translating the radiant energy of the sun into adenosine triphosphate (ATP). In animals, this occurs by converting substrate fuels into energy through the process of oxidative phosphorylation (OXPHOS) that occurs exclusively in mitochondria (Figure 1). These organelles catabolize glucose- and amino acid–derived carbon molecules and long-chain fatty acids to acetyl coenzyme A (CoA), which promotes flux through the tricarboxylic acid (TCA) cycle. Reducing equivalents, generated by various mitochondrial dehydrogenase reactions, provide electrons that are utilized by the first four complexes of the respiratory chain to reduce molecular oxygen to water. The fifth complex capitalizes on the proton gradient across the mitochondrial membrane generated by electron transport to drive the synthesis of ATP from adenosine diphosphate (ADP) and inorganic phosphate, thus completing OXPHOS. Because OXPHOS yields the vast majority of high-energy phosphate molecules to sustain myriad intracellular reactions, interruption of mitochondrial fuel metabolism can be disastrous for the host.

Central to the efficiency of OXPHOS is the metabolic fate of pyruvate, which is produced in the cytoplasm, mainly by glycolysis, and which exists in equilibrium with alanine and lactate. In addition to its interconversion with other 3-carbon molecules, pyruvate may also be carboxylated to oxaloacetate by the anaplerotic reaction catalyzed by pyruvate carboxylase or may be decarboxylated to acetyl CoA by the multienzyme pyruvate dehydrogenase complex (PDC). Many of the calories we consume daily pass through the PDC, which, under aerobic conditions, catalyzes the rate-determining step of glucose and

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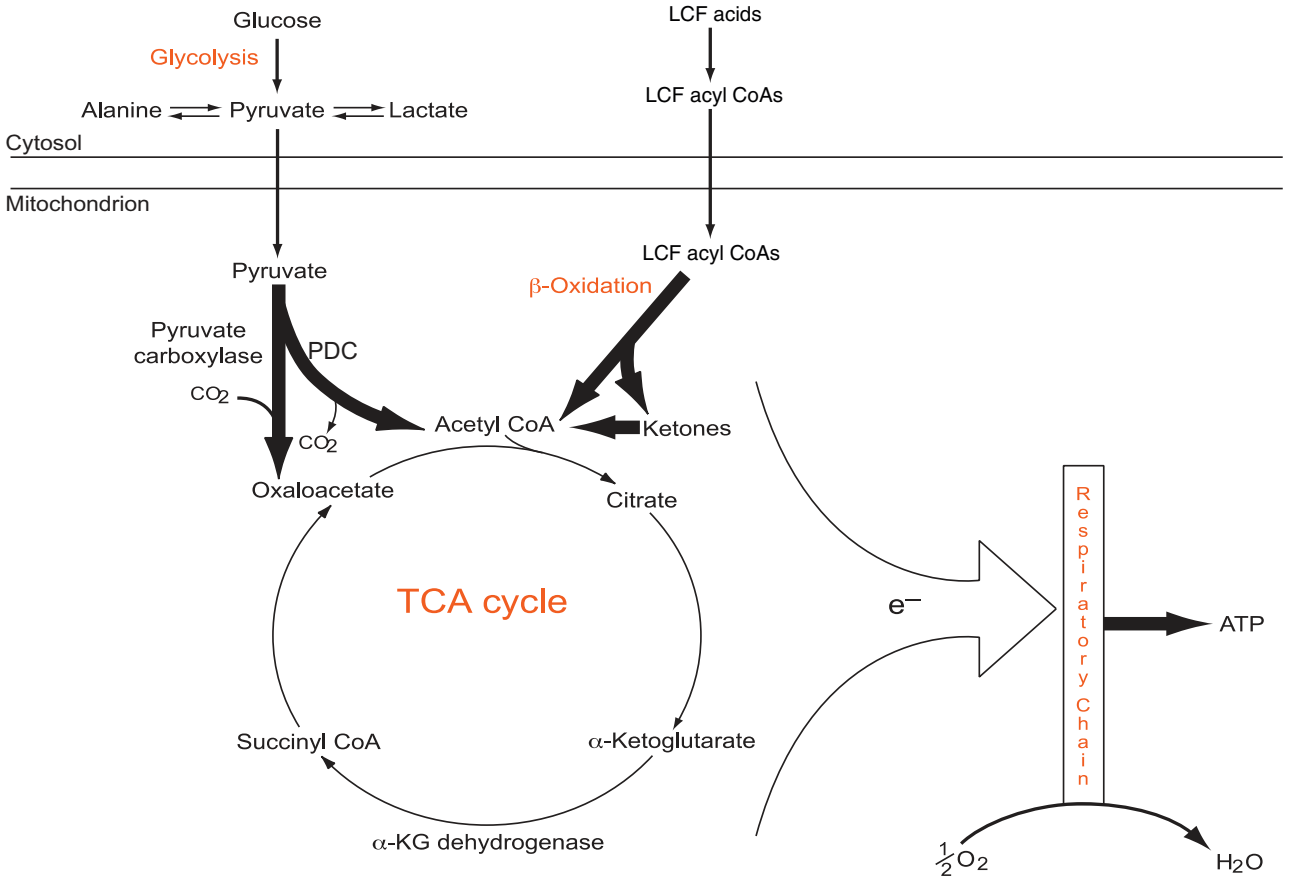


Figure 1. Pathways involved in mitochondrial energetics. The two principal mitochondrial fates of glycolytically derived pyruvate are to be carboxylated to oxaloacetate, an anaplerotic reaction catalyzed by pyruvate carboxylase, or to be decarboxylated to acetyl coenzyme A (CoA) by the pyruvate dehydrogenase complex (PDC), which condenses with oxaloacetate to form citrate. Thus, PDC links cytoplasmic glycolysis to the mitochondrial tricarboxylic acid (TCA) cycle. Long-chain fatty acids may undergo beta-oxidation to also yield acetyl CoA. Reducing equivalents (NADH, $FADH_2$) generated by the PDC catalyzed step, beta-oxidation, and various dehydrogenases in the TCA cycle, such as alpha ketoglutarate dehydrogenase, donate electrons that enter the respiratory chain of NADH ubiquinone oxidoreductase (complex I) or at succinate ubiquinone oxidoreductase (complex II). Cytochrome c oxidase (complex IV) catalyzes the reduction of molecular oxygen to water and adenosine triphosphate (ATP) synthase (complex V) produces ATP from ADP and inorganic phosphate. ATP = adenosine triphosphate; ADP = adenosine diphosphate; CoA = coenzyme A; e- = electrons; LCF = long-chain fatty acid; PDC = pyruvate dehydrogenase complex; TCA = tricarboxylic acid.

pyruvate oxidation. The PDC and acyl CoA carboxylase, which initiates the beta-oxidation pathway of long-chain fatty acids, provide the intramitochondrial starting points for OXPHOS. Thus, it is not surprising that loss of function mutations in any component of the PDC or post-translational inhibition of the enzyme can lead to devastating clinical consequences (1).

Structure and Regulation of the Mammalian PDC

The 9.5M Da PDC megacomplex is organized into multiple copies of three enzymatic components (Figure 2) (2–4). The PDC is highly regulated transcriptionally and post-transcriptionally (5–8). Changes in the enzymatic activity of the complex are mediated primarily by reversible phosphorylation, in which phosphorylation of any one of three serine residues (site 1, Ser 293; site 2, Ser 300; site 3, Ser 232) on the alpha subunit (E1α) of the E1 (pyruvate dehydrogenase) component renders the entire complex inactive. Phosphorylation of PDC is catalyzed in humans by any of four isoforms of pyruvate dehydrogenase kinase (PDK1-4) that exhibit 70% homology. PDKs are serine/threonine kinases that are tightly associated with the PDC and bind to the L2 domain of E2, with the following relative binding affinities: PDK3 > PDK1 ≃ PDK2 > PDK4 (9). Each PDK exhibits different specificities, in terms of phosphorylation of E1α and rates of phosphorylation (site 1: PDK2 > PDK4 ≃ PDK1 > PDK3; site 2: PDK3 > PDK4 ≃ PDK2 > PDK1) (10). Two pyruvate dehydrogenase phosphatase isoforms (PDP1 and 2) dephosphorylate the E1α subunit and restore catalytic activity. PDPs are dimers of catalytic (52 kDa) and regulatory (96 kDa) subunits that are differentially regulated by certain divalent cations and polyamines (5,11). Recent evidence suggests that PDPs may be post-translationally modified in some cancers (12,13). However, the current understanding of the relationship between PDPs and cancer is nascent, and neither PDP isoform has yet been investigated as a potentially "drugable" target for any disease.

PDKs show variable tissue expression and sensitivity toward regulation by a diverse array of endogenous molecules and xenobiotics (5–7,14–17). Rapid regulation of PDC activity is mediated by substrate activation and end product inhibition. PDK2 has the widest tissue distribution and is particularly sensitive to activation by the PDC reaction products acetyl CoA and NADH. Acetyl CoA enhances acetylation of the reduced lipoyl groups by the E2 subunit of PDC. NADH accumulation induces modest

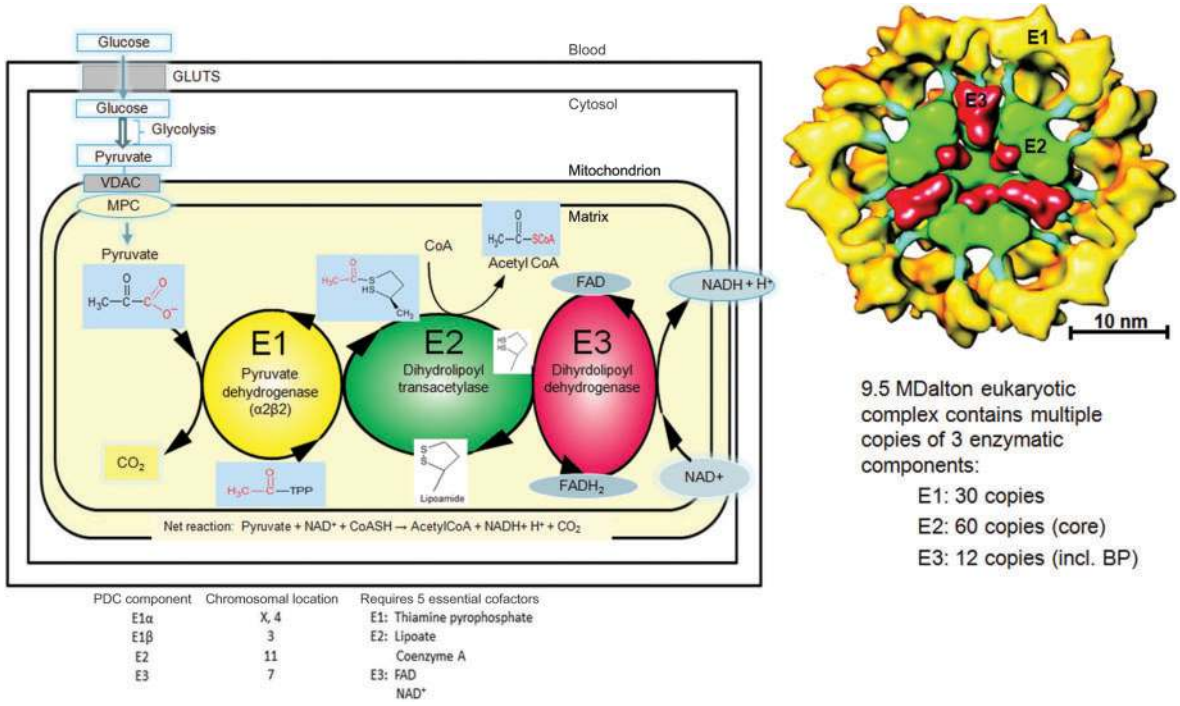


Figure 2. Pyruvate dehydrogenase complex (PDC mechanism and assembly). Glucose enters cells through glucose transporters and is metabolized to pyruvate in the cytoplasm. Pyruvate crosses the outer mitochondrial membrane by means of voltage-dependent anion channel and the inner mitochondrial membrane via the mitochondrial pyruvate carrier system. Pyruvate is irreversibly decarboxylated by the E1α subunit of the heterotetrameric (x_2p_2) pyruvate dehydrogenase enzyme, the first component of the multienzyme PDC. E2 (dihydrolipoyl transacetylase) transfers the acetyl group to a lipoic acid moiety that synthesize up to 60 molecules of acetyl coenzyme A (CoA) from reduced CoA per macromolecular complex. Lipoate is reoxidized by the E3 component (dihydrolipoamide dehydrogenase) in a coupled redox reaction that yields NADH. PDC also utilizes an E3 binding protein to tether the E3 component to the complex's core. The E1α subunit is located on the X chromosome, while the remaining PDC proteins are nuclear encoded. Multiple cofactors are required to enable each enzymatic step of the net PDC-catalyzed reaction. Assembly structure of PDC from reference 2. GLUTS = glucose transporters; PDC = pyruvate dehydrogenase complex; VDAC = voltage-dependent anion channel;

(20%–30%) increases in PDK1 and PDK2. However, an increase in both acetyl CoA and NADH, as occurs in mitochondrial beta-oxidation of long-chain fatty acids, results in a two- to threefold stimulation of PDK1 and PDK2 activities (18).

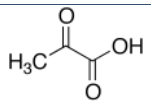
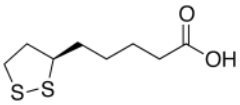
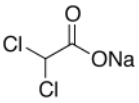
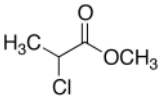
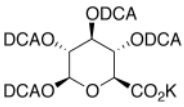
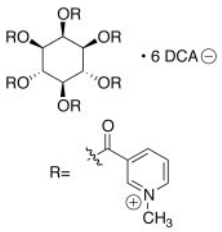
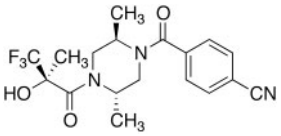
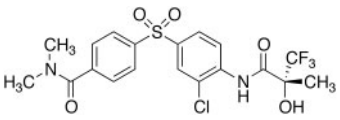
The N-terminal domain of PDK contains a small binding site for pyruvate, which is both the natural PDK inhibitor and PDC substrate, and for the xenobiotic pyruvate analog dichloroacetate (DCA). The amino-terminal domain also possesses binding pockets for other synthetic PDK inhibitors AZ12, Nov3r, Pfz3, and AZD7545 (Table 1) (19). The carboxy terminal domain includes the nucleotide binding site and an interface that allows dimerization of the protein, with the active site of PDK thought to be located on the domain's interface (18–21). Potassium ions appear to be essential for optimal nucleotide binding to PDK2 and also facilitate binding of PDK2 to the inner lipoyl-bearing domain of the E2 component (dihydrolipoamide transacetylase) of PDC (19).

In addition to phosphorylation, PDC may be reversibly acetylated (22), glutathionylated (23), succinylated (24,25), and glycosylated (26), although the relative physiological impacts of these post-translational changes remains to be clarified. Recently, the sirtuin SIRT4 was found to possess lipoamidase activity, enabling hydrolysis of the lipoamide cofactors from the E2 component and resulting in reduced activity of PDC (27). This finding is potentially physiologically highly relevant, given the importance of the mitochondrially localized SIRTs 3-5 in regulating cellular energetics, signaling, and apoptosis (28). Moreover, glutamine, an important energy substrate in cancer and other proliferative conditions (29), can induce SIRT4 lipoamidase activity, providing a mechanism for the well-established inhibition of PDC in cancer (reviewed in [30]). Finally, PDC was recently discovered to be capable of exiting the mitochondria and imported, intact and functional, into the nucleus (31), thus considerably extending the biological spectrum of this remarkable enzyme megacomplex.

Metabolic Flexibility, the PDC/PDK Axis, and Cancer

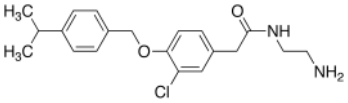
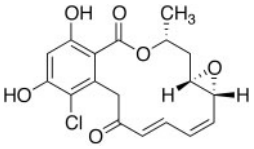
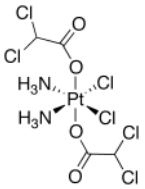
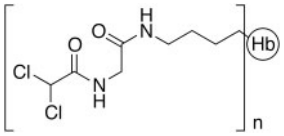
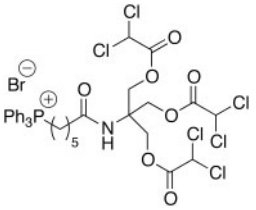
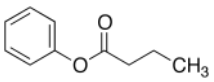
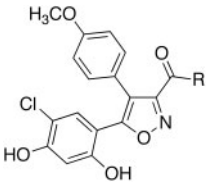
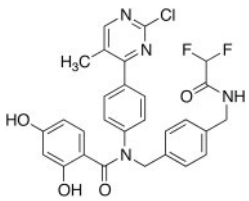
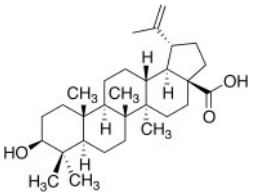
Metabolic flexibility may be defined as the ability of a cell to adaptively utilize metabolic pathways in order to maintain energy status and physiological functions. Such reprogramming has emerged as a hallmark of the physiological and pathological alterations affecting cellular function and survival. Differentiation of the developing conceptus (32–35), differentiation of pluripotent stem cells (36–39), wound healing (40), and activation of the innate immune inflammatory response (41–44) are but some of many homeostatic and defense processes requiring metabolic reprograming necessary for organismal development, repair, and survival. Indeed, the dynamic control of metabolism by immune cells in response to invading pathogens illustrates the critical importance of metabolic flexibility to survival. Inhibition of PDC activity has been causally associated with many acquired disorders, including lactic acidosis (45), diabetes and other insulin-resistant states (17,46–48), cerebrovascular and cardiovascular diseases (49–51), late-onset neurodegenerative diseases (52–54), cancer (30,55), pulmonary

Table 1. Small molecule pyruvate dehydrogenase Kinase PDK inhibitors*

Chemical name	Structure	Properties	Reference
Pyruvate		Ki PDK2 < PDK1≃ PDK4 < PDK3; uncompetitive inhibitor; synergizes with ADP; IC_{50}:mM range	Stacpoole et al. 1987 (174)
R-lipoic acid		Ki PDK1 < PDK4 PDK2 < PDK3; IC_{50}:mM range	Korotchkina et al. 2004 (101)
Dichloroacetate (sodium salt)		Allosteoric inhibitor; synergizes with ADP; Ki PDK2 < PDK 1≃ PDK4 < PDK3; IC_{50}:mM range	Whitehouse et al. 1974 (103)
2-chloroproprionate		Similar in site of action and potency to DCA	Whitehouse et al. 1974 (103)
Inositol esters or ionic complexes, eg, inositol hexa (N-methylnicotinate-dichloroacetate) and tetra (dichloroacetyl) gluconate (potassium salt)	 Potassium tetra (dichloroacetyl) glucuronate  Inositol hexa (N-methynlicotinate-dichloroacetate	Designed to achieve slow release of DCA, reducing peak plasma drug levels and prolonging dynamics; similar to DCA in dynamics but more toxic; IC_{50}:mM range	Stacpoole et al. 1987 (174)
(R)-3.3.3-trifluoro-2-hydroxy-2-methyl propionamides, eg, Nov3r (Novartis)		First orally active PDK inhibitors not based on α-dihalo-genated carbonyl compounds (eg, DCA. 2-CP); binds to lipoyl group binding site at N terminus; IC_{50}:mM range	Aicher et al. 1999a (136) Aicher et al. 1999b (137)
Anilide tertiary carbinols, eg, AZD7545 (AstraZeneca)		Inhibits PDK1-3 by binding to lipoyl group binding site; IC_{50}:mM range	Bebernitz et al. 2000 (138)

(continued)

Table 1. (continued)

Chemical name	Structure	Properties	Reference
N-(2-aminoethyl)-2{3-chloro-4-[(4-isopropylbenzyl)oxy] phenyl}acetamide; Pfz3 (Pfizer)		Allosteric PDK2 inhibitor; IC_{50}:mM range	Knoechel et al. 2006 (105)
Radicicol (monorden)		Binds to ATP binding pocket of PDK3; IC_{50}:mM range	Besant et al. 2002 (175)
Mitaplatin		Fusion molecule of 1 cisplatin: 2 DCAs via ester linkages; IC_{50}:mM range	Xue et al. 2012 (176)
Hemoglobin-DCA conjugate		Fusion molecule of 1 Hgb: 12 DCAs targeting monocytic leukemic cells; IC_{50}:mM range	Zhang et al. 2011 (157)
Mito-DCA		Fusion molecule of 1 triphenyl-phosphonium cation: 3 DCAs to reportedly facilitate transport across mitochondrial membrane; IC_{50}:mM range	Pathak et al. 2014 (154)
Phenylbutyrate		Binds to allosteric site (PDK 1-3 only); IC_{50}:mM range	Iannitti et al. 2011 (155) Ferriero et al. 2015 (156)
4,5-diarylisoxazoles		Bind to ATP binding pocket; IC_{50}:mM range	Meng et al. 2014 (146)
VER-246608		Pan-PDK isoform inhibitor at ATP binding site; IC_{50}:mM range	Moore et al. 2014 (147)
Betulinic acid		Fusion molecule of betulinic acid to DCA; IC_{50}:mM range	Saha et al. 2015 (118)

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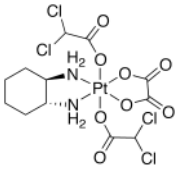
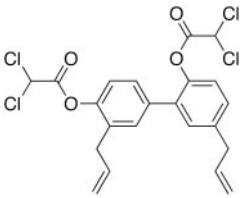
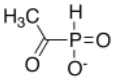
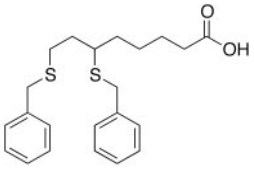
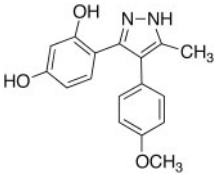
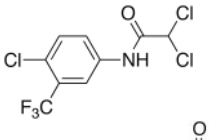
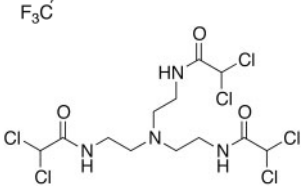
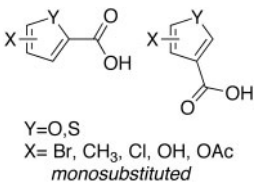
REVIEW

Table 1. (continued)

Chemical name	Structure	Properties	Reference
DCA-oxaliplatin derivatives		Fusion molecule of 1 platin: 2DCAs via ester linkages; IC_{50}:mM range	Liu et al. 2015 (151)
Honokiol DCA (ester derivative from Magnolia grandiflora)		Reported to increase DCA lipophilicity; IC_{50}:mM range	Bonner et al. 2016 (177)
Pyruvate analogs containing phosphinate or phosphonate group (eg, acetyl phosphinate)		Competitive inhibitor of pyruvate; IC_{50}:mM range	Meurs et al. 2012 (161)
CPI-613 (lipoate derivative)		Inhibits lipoate interactions; IC_{50}:mM range	Zachar et al. 2011 (158)
M77976 (dihydroxyphenyl pyrazole derivative)		Binds to PDK4 ATP pocket; IC_{50}:mM range	Hiromasa et al. 2006 (98)
Aromatic DCA derivatives		Binds to PDK1 ATP pocket; IC_{50}:mM range	Zhang et al. 2016 (153)
DCA-loaded tertiary amines		Reported to increase DCA stability; IC_{50}:mM range	Trapella et al. 2016 (152)
Furan and thiophene carboxylic acids		Allosteric pyruvate site binding PDK2 inhibitors	Masini et al. 2016 (159)

*2-CP = 2-chloroproprionate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; DCA = dichloroacetate; GBM = glioblastoma; PDC = pyruvate dehydrogenase complex; PDK = pyruvate dehydrogenase kinase.

arterial hypertension (56,57), and aging (58). The most common underlying mechanism accounting for PDC inhibition in these conditions is post-transcriptional upregulation of one or more PDKs, leading to phosphorylation of the E1α subunit of PDC. Such perturbation of the PDC/PDK axis induces a "glycolytic shift," whereby affected cells favor ATP production by glycolysis over OXPHOS (Figure 3). Indeed, over 80 years ago, Otto Warburg noted the propensity of tumor cells to preferentially utilize glycolysis, rather than mitochondrial oxidation, for energy production, even in the presence of adequate oxygen concentrations (59). A molecular basis for this phenomenon, known as aerobic glycolysis, or the Warburg effect, has emerged over the past

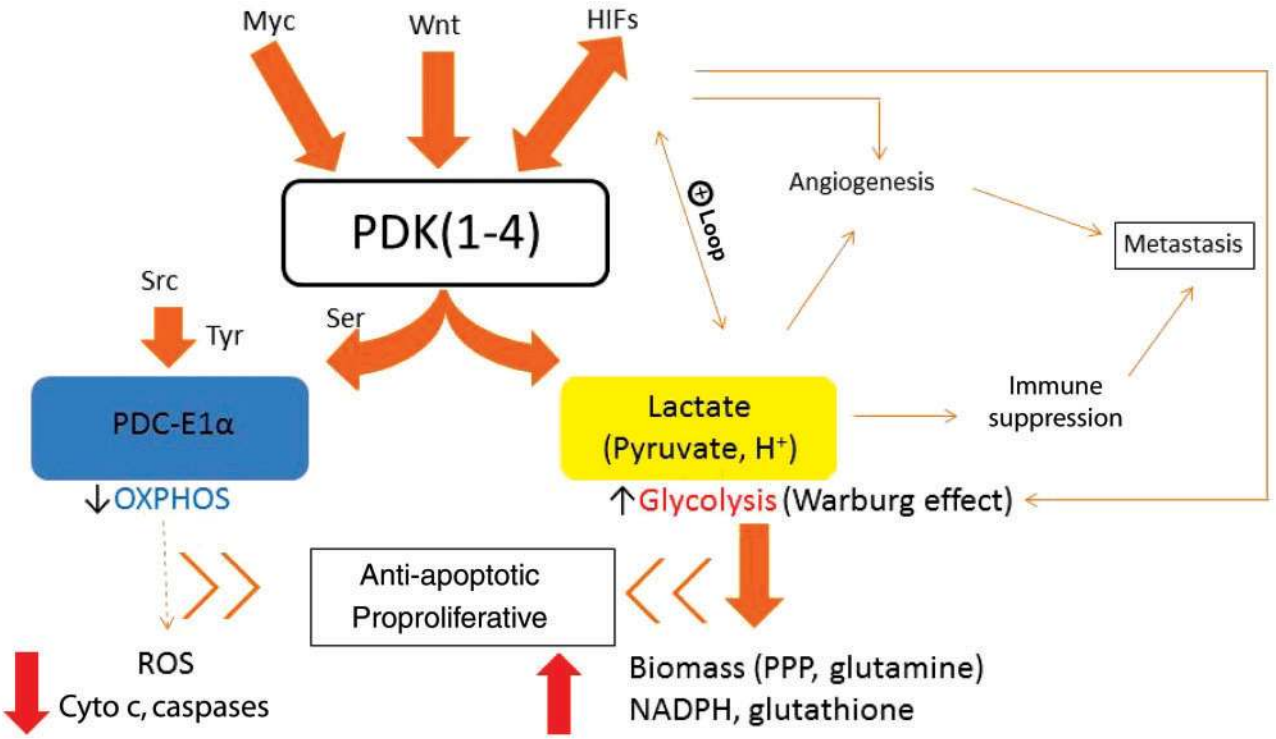


Figure 3. Mechanisms regulating the pyruvate dehydrogenase complex/pyruvate dehydrogenase kinase (PDC/PDK) axis in cancer. Multiple transcription factors, such as Myc, Wnt, and hypoxia inducible factors (HIFs) act singly or in concert to transcriptionally increase one or more pyruvate dehydrogenase kinase (PDK) isoforms in a cancer cell. Activated PDK phosphorylates one or more serine residues on the E1α subunit of the pyruvate dehydrogenase complex (PDC) and inhibits its activity and pyruvate-driven oxidative phosphorylation (OXPHOS). In turn, reduced OXPHOS activity decreases reactive oxygen species production from the respiratory chain and the production of other mitochondrially derived chemical signals associated with the induction of apoptosis. In addition, the oncogene Src is reported to directly phosphorylate tyrosine residues on E1α in some cancers independently of any changes in PDK expression. Inhibition of PDC and upregulation of glucose transporter 1 and glycolytic enzymes by HIF and other transcription factors combine to increase intratumoral pyruvate, lactate, and hydrogen ions, which stabilize HIF, thus creating a positive feedback. This metabolic remodeling induces a high glycolytic rate in tumor cells under both hypoxic and normoxic conditions, the latter resulting in aerobic glycolysis (Warburg effect). Lactate also suppresses immune surveillance, helping drive tumor metastasis. Accelerated glycolysis also shunts the glycolytic intermediate glucose-6-phosphate to the pentose phosphate pathway (hexose monophosphate shunt), thereby supplying reducing equivalents (nicotinamide adenine dinucleotide phosphate) and carbon for synthesis of glutathione and nucleotides, buffering tumor cells against oxidative stress and providing biomass for new cells. Many tumors also draw heavily on glutamine and pyruvate carboxylase-mediated conversion of pyruvate to oxaloacetate for anaplerotic maintenance of the tricarboxylic acid cycle. HIF = hypoxia inducible factor; NADPH = nicotinamide adenine dinucleotide phosphate; PDC = pyruvate dehydrogenase complex; PDK = pyruvate dehydrogenase kinase; PPP = pentose phosphate pathway; ROS = reactive oxygen species.

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decade. Stable overexpression of the master transcription factors hypoxia-inducible factor 1 alpha (HIF1α) and the oncogene Myc, among others, plays a determining role in the pathogenesis of the Warburg effect. The relationship between HIF1α and cancer biology is being intensely investigated (60,61), but actions of HIF1α most relevant to this discussion are the upregulation of the widely expressed plasma membrane glucose transporter GLUT1, most enzymes of glycolysis, and all PDKs. As a result, PDC and OXPHOS are inhibited, and the rates of glycolysis and lactate production are accelerated. Indeed, intratumoral lactate concentrations may reach 10 to 20 mM and have been inversely associated with tumor recurrence, proliferation, and survival (62–64). Magnetic resonance imaging studies of glioblastomas (GBMs) have also revealed high lactate concentrations relative to normal brain tissue and an inverse association between tumor lactate (but not pyruvate) and survival (65–67). The relationship between lactate and cancer progression reflects the pleotropic actions of this three carbon molecule. Multiple independent studies have demonstrated that lactate generated by hypoxic tumor cells reaches oxidative tumor cells via the monocarboxylate transporter 1, where it is utilized as an important mitochondrial energy substrate (68–71). By its metabolism, lactate acts as a free radical quencher to limit oxidative stress in tumor cells and induce tumor radioresistance (72–74). Lactate also modulates immune cell function to suppress host immunosurveilance and promote tumor cell migration and metastasis (75–78). Furthermore, lactate helps sustain a positive feedback loop, whereby lactate, pyruvate, hydrogen ions, and the TCA cycle intermediates succinate and fumarate can stabilize HIF1a, perpetuating upregulation of glycolytic enzymes, PDKs, and various angiogenesis-stimulating molecules to facilitate tumor growth and survival (70,79,80). Consequently, lactate per se has become a potential therapeutic target in cancer, either by inhibiting its uptake and/or formation by tumor cells (71,77,81), or by accelerating its oxidative removal at the level of the PDC/PDK axis (30,82). The glycolytic shift also results in disruption of cellular ion channels, increased mitochondrial membrane potential ($\Delta\Psi m$), and multiple additional biochemical events that promote tumor growth, metastasis, and survival. PDK1 and 4, which are normally most highly expressed in heart and skeletal muscle, respectively, in healthy organisms, are also frequently overexpressed in diverse cancers (15,83–85) and have been associated with the capacity of some tumors to resist anoikis (86). PDK3 expression in colon cancer is also reported to be associated directly with metastatic spread and inversely with survival (87). Thus, specific PDK isoform expression in

tumors cannot always be predicted from expression levels found in normal tissues. There is also evidence that PDKs may at least indirectly influence cell cycle events (84) and can, in turn, be regulated by oncogenes (85–87).

Targeted inhibition of PDKs reverses the Warburg effect in tumor cells, reduces lactate concentration in the tumor microenvironment, increases the production of mitochondrially generated reactive oxygen species (ROS), and decreases $\Delta\Psi m$ and HIF1α expression and induces a caspase-mediated apoptosis selective to tumor cells (88), leading to decreased tumor vasculogenesis and proliferation in vivo and increased survival in several experimental models (reviewed in [30]).

Solid tumors typically are heterogeneous collections of both malignant stem and differentiated cells and of host stromal cells, with varying degrees of oxygen tension, vascularity, and bioenergetic needs (89–92). Furthermore, the relationship between aerobic glycolysis and oxidative metabolism in tumors is not controlled by a toggle switch but by a rheostat, enabling both processes to occur concurrently to variable degrees. Such plasticity in the regulation of metabolism yields multiple benefits to a cancer's survival and growth. Aerobic glycolysis per se produces modest amounts of ATP compared with OXPHOS. However, acidification of the tumor microenvironment by increased production of lactate and hydrogen ions has been linked directly to host immune suppression, tumor invasion, and decreased host survival (reviewed in [30]). Accelerated glycolysis by cancer cells also provides increased glucose carbon via glucose 6-phosphate to the pentose phosphate pathway (hexose monophosphate shunt), which supplies nucleotide precursors and reduced nicotinamide adenine dinucleotide phosphate (NADPH) for glutathione synthesis. Thus, glycolysis generates both tumor biomass and protection against oxidative stress.

Upon entering the mitochondrial matrix, pyruvate may be irreversibly decarboxylated by PDC to acetyl CoA or irreversibly carboxylated by pyruvate carboxylase (PC) to oxaloacetate (Figure 1). Overexpression of PDK by tumor cells would inhibit PDC and thus potentially redirect pyruvate to oxaloacetate formation via pyruvate carboxylase, which catalyzes an anapleurotic process aimed at maintaining TCA cycle intermediates. Such a mechanism has been described in non–small cell lung carcinomas in vivo, from data obtained by perioperative administration of uniformly labeled ^{13}C-glucose (93). However, active flux through PDC and robust TCA cycle activity in vivo has also been reported using stable isotope kinetics in patients with glioblastomas (94). Another anaplerotic substrate of likely importance in tumor metabolism is glutamine, which is converted to the TCA cycle intermediate α-ketoglutarate in mitochondria by glutaminase (95). Regardless of how the TCA cycle is maintained, it provides multiple anabolic precursors critical for biomass production and growth of tumors.

Small Molecule PDK Inhibitors

As recently reviewed (96,97), the known PDK inhibitors act at one of four binding sites: 1) a pyruvate binding site, 2) a nucleotide binding site, 3) a lipoamide binding site, and 4) an allosteric site. All but the nucleotide binding pocket are located in the regulatory N-terminal R domain of the kinases (98).

Naturally Occurring Inhibitors

Several endogenous molecules can modify PDC activity (Table 1). As described above, pyruvate, NAD^+, and CoA exert substrate activation of PDC by inhibiting PDKs, although only pyruvate acts on the kinases directly, while the stimulatory effects of NAD^+ and acetyl CoA are mediated via reductive acetylation of the lipoyl residues of the inner lipoyl domains of the E2 component (dihydrolipoyl acetyl transferase), thereby inhibiting docking of PDK to PDC (20). Substrate-level doses of pyruvate increase oxygen consumption in certain tumors, inducing transient hypoxia and potentiating the antitumor effects of the hypoxia-activated prodrug TH-302 (99,100). Naturally occurring R-lipoic acid is covalently attached to the PDC through a specific lysine residue to facilitate the overall decarboxylation of pyruvate to acetyl CoA. R-lipoic acid and, to variable degrees, S-lipoic acid and R-dihydrolipoic acid stimulate PDC activity by inhibiting PDKs, although the precise molecular mechanisms are unknown (101).

Dichloroacetate

The original xenobiotic developed for clinical use as a PDK inhibitor is DCA, which was discovered to modulate glucose and fat metabolism (102) before its direct effect on the PDC/PDK axis was known (103). DCA is most active against the ubiquitous PDK2 isoform (Ki ~0.2 mM), approximately equipotent against PDK1 and PDK4, and has low activity against the PDK3 isoform that normally is most abundant in testes (5). DCA is thought to bind to the pyruvate-binding pocket, together with ADP, leading to disruption of the binding of the kinase to the lipoyl (E2) domain of PDC (104). Other evidence suggests that DCA binds to the allosteric site to promote local conformational changes in both the nucleotide and lipoamide binding sites (96,105,106). DCA is rapidly absorbed, widely distributed in vivo, and readily crosses the blood-brain barrier, which accounts for the rapid (within minutes) stimulation of PDC activity following its oral or parenteral administration (reviewed in [49]). Repeated dosing leads to a more sustained increase in PDC activity that has been attributed to decreased enzyme turnover (reviewed in [107]), thereby providing a second mechanism for PDC stimulation.

DCA has been administered as an investigational drug for over 30 years in the treatment of type 2 diabetes, acquired and congenital hyperlipoproteinemias, myocardial ischemia and failure, acquired and congenital lactic acidosis (due to PDC deficiency and other inborn errors of mitochondrial metabolism), and, most recently, cancer (30,55,107). Typical oral and parenteral daily doses range from 10 to 50 mg/kg. Its effect on PDC activity occurs within 15 to 30 minutes of an administered dose, as observed clinically by the reduction in circulating lactate concentration, which is a useful biomarker of the drug's in vivo dynamics (49,108). Early concerns about DCA's toxicity in rodents and canines (49) were not reproducible in chronically treated humans, except for a reversible sensory and motor peripheral neuropathy (109). However, this adverse effect appears dependent mainly on the age and genotype of the recipient (107). Biotransformation of DCA to glyoxylate (which is inactive toward PDC) is mediated by glutathione transferase zeta 1 (GSTZ1), with the rate of biotransformation directly dependent on subject age (110) and *GSTZ1* haplotype expression (111). The drug is safe when administered daily for one to two decades in children with congenital PDC deficiency or other primary mitochondrial diseases (112) and is generally well tolerated in adults, providing dose adjustments are made for age and *GSTZ1* haplotype.

Since the first report in 2007 of DCA's pro-apoptotic effects in human cancer cells studied in vitro and in animals implanted with human tumors (88), more than 200 preclinical studies of human tumors derived from all three germ layers have been fairly consistent in reporting the ability of DCA to knock down

Table 2. The PDC/PDK axis as a therapeutic target in brain cancer*

Study design	Major findings	Reference
Cell culture	First demonstration of antitumor effect of small molecule targeting (DCA); PDC/PDK axis in multiple human tumors, including glioblastoma	Bonnet et al. 2007 (88)
Cell culture; xenograft	DCA reversed Warburg effect in rat glioma cells and synergized with etoposide or irradiation to induce Foxo 3 and p53 and Bax-dependent apoptosis	Morfouace et al. 2012 (165)
Cell culture; xenograft	DCA reversed Warburg effect and decreased proliferation of human GBM xenograft	Li et al. 2015 (116)
Cell culture; xenograft	DCA reversed Warburg effect and Hedgehog-dependent neuronal and meduloblastoma growth	Di et al. 2014 (166)
Cell culture	DCA reactivated the suppressed PDC activity and abrogated clonogenic advantage in u87 GBM cells conferred by roH1 mutation and decreased proliferation in vitro	Izquierdo-Garcia et al. 2015 (167)
Cell culture	DCA induced mitochondrial-mediated oxidative stress and apoptosis in multiple GBM cell lines and primary cultures	Shen et al. 2015 (168)
Cell culture; xenograft	DCA induced cell cycle arrest and sensitized GBM cells in vitro and in vitro to irradiation, increasing survival	Shen et al. 2015 (169)
Cell culture	DCA induced cell cycle in human GBM cells	Takakashi et al. 2015 (170)
Cell culture; xenograft	DCA inhibited growth of GBM cells and increased survival of rodent glioma allograft	Wicks et al. 2015 (171)
Allograft	Hyperpolarized [2-^{13}C pyruvate] used in rat glioma model to demonstrate DCA's ability to promote oxidative metabolism in tumor	Park et al. 2015 (172)
Allograft	Hyperpolarized [1-^{13}C pyruvate] used in rat glioma model to demonstrate DCA's ability to increase flow through PDC	Park et al. 2013 (173)
Excised GBM and phase I trial	DCA inhibited H1F1x, PDK, and angiogenesis, depolarized mitochondria, increased tumor apoptosis, and caused reversible peripheral neuropathy and possibly decreased tumor growth in 5 adults with recurred GBMs	Michelakis et al. 2010 (133)
Phase I trial	Personalized, genetics-based DCA dosing in 8 adults with recurred brain tumors was well tolerated, without major neurotoxicity	Dunbar et al. 2014 (113)

*DCA = dichloroacetate; GBM = glioblastoma; PDC = pyruvate dehydrogenase complex; PDK = pyruvate dehydrogenase kinase.

the levels of overexpressed tumor PDKs, shift metabolism from glycolysis to OXPHOS, depolarize the $\Delta\Psi m$, and, when measured, decrease tumor volume, proliferation, and metastases (30,113–122). Particular attention has focused on the drug's preclinical and clinical effects in brain cancer (Table 2). Like pyruvate, DCA has been shown to stimulate tumor oxygen consumption, inducing a local hypoxic state that increases tumor sensitivity to hypoxia-specific chemotherapy (123). However, resistance to the in vitro antitumor effects of DCA has also been reported and appears to be due to variable molecular mechanisms associated with specific tumor cell types (30,124–126). More consistent are the drug's in vivo actions against human cancer xenographs in rodent models. Stable upregulation of HIF1α in tumors not only exerts a metabolic shift favoring glycolysis over OXPHOS and inhibition of release from mitochondria of pro-apoptotic factors, but also promotes tumor proliferation and metastases by increasing expression of vascular endothelial growth factor (VEGF) and other vasculogenic molecules (127). The impact of HIF1α-induced vasculogenesis is best appreciated experimentally by in vivo experiments, in which DCA inhibits HIF1α expression, tumor vasculogenesis, and proliferation and increases survival (reviewed in [30]).

An additional property of the drug that may have therapeutic potential in cancer is inhibition of mitochondrial fatty acid β-oxidation, which, so far, has been established only in mammalian muscle and liver under conditions of fasting or diabetes (122,128). Long-chain fatty acid oxidation has recently been found to be essential for nucleotide and DNA synthesis in endothelial cells, and inhibition of β-oxidation blocks endothelial cell proliferation (129). Thus, in addition to decreasing HIF1α-mediated angiogenesis, DCA may exert another anti-angiogenic action through suppression of a metabolic process required for endothelial cell growth.

DCA may exhibit another potential mechanism of antitumor action because of its effect on lactate metabolism. DCA is the most potent lactate-lowering drug in clinical use (108) because of its ability to stimulate PDC activity and increase oxidative removal of lactate. The ability of DCA to lower tissue and circulating levels of lactate (and accompanying protons) may be an underappreciated, but potent, antitumor action of this and other PDK inhibitors because of the role of lactate in tumor immunity, growth, and metastasis and the well-established inverse clinical association of lactate with survival (30,130–132).

Two phase I trials of oral DCA (≤25 mg/kg/d) in adult patients with recurrent primary high-grade astrocytomas or metastases to the brain from non–central nervous system cancers (113,133), one phase I trial in adults with various solid tumors (55), and a brief report of three other patients with recurrent malignancies (134) have indicated the drug to be generally well tolerated. Peripheral neuropathy remains a dose-limiting side effect of chronic DCA (55,133), but it can be mitigated or prevented by exploiting genetics-based dosing (113).

DCA Mimetics

Many other mono- or di-halogenated derivatives of short-chain fatty acids have been found to activate PDC by inhibiting PDKs

and probably also bind to the pyruvate site (103). Of these, 2-chloropropionate is similar in potency to DCA in rat heart mitochondria, but it is too toxic for clinical use (135). DCA is not patentable, so its development has been hampered by a lack of pharmaceutical support. However, as the prototypic PDK inhibitor, DCA has generated considerable interest from industry and academia, resulting in the synthesis of many patentable small molecule derivatives designed to be more potent and/or more selective toward PDKs. Early efforts by Novartis (136–139) and AstraZeneca (105,139–142) to develop inhibitors for the treatment of type 2 diabetes failed because of lack of efficacy, untoward toxicity, or both (143). It is noteworthy that nearly all of these derivatives contain one or more halogens (chloride and/or fluoride) attached to much larger and bulkier structures than DCA and bind to sites other than that occupied by pyruvate or DCA (Table 2). Others (144) have employed three-dimensional quantitative structure-activity relationships using comparative molecular field analysis to design a number of small molecule inhibitors that, to date, lack published evidence of safety or biological activity.

The most recent burst of interest in developing PDK inhibitors is founded on DCA's reported anticancer effects. Many compounds have been advocated as therapeutic "metabolic modulators" of cancer cells, targeting glucose uptake, glycolysis, mitochondrial respiration, $\Delta\Psi m$, the mitochondrial permeability transition pore, or mitochondrial DNA (147). Numerous preclinical studies have employed DCA as combination therapy with established anticancer agents. Newly synthesized anticancer PDK inhibitors include Mitaplatin (148–150), which contains two molecules of DCA bound to cisplatin. Mitaplatin dissociates within cells, allowing cisplatin to target nuclear DNA while DCA inhibits PDK and reverses the Warburg effect. Although initial preclinical studies are promising, it will be interesting to observe the chronic safety and tolerability of combining these two potential peripheral neurotoxins. Others have synthesized diam(m)ine platinum (II) complexes also bearing DCA, the most potent in vitro being an equimolecular combination of DCA and the oxaliplatin analog (151).

Betulinic acid (BA) is a pentacyclic triterpenoid that occurs naturally in some plants and that is reported to have antitumor activity but poor solubility (118). Esterification of the C-3 hydroxyl group of BA with DCA creates "Bet-CA," which increases the solubility of BA and is reported to reduce tumor growth and metastases to an extent greater than either BA or DCA alone. DCA-loaded tertiary amines have been synthesized, ostensibly to increase DCA in vivo stability (152), as have aromatic derivatives (153).

Multiple DCA molecules have also been bound to hemoglobin (97). The complex is thought to be taken up by cells by the hemoglobin scavenger receptor, with the desired purpose of treating monocytic leukemia. So-called "mito-DCA" links a lipophilic trephenyl-phosphorium cation to three DCA molecules and has been reported to exceed DCA's in vitro activity toward human prostate cancer cells (154).

Phenylbutyrate also stimulates PDC by inhibiting PDKs; its potency is similar to that of DCA, but its site of inhibition differs. The drug is already in use for certain rare urea cycle disorders, but it has multiple other pharmacological actions, including inhibition of histone deacetylation (155,156). Phenylbutyrate so far lacks published evidence of safety and efficacy in conditions in which there is a congenital or acquired defect of PDC.

PDK isoforms belong to the GHKL ATPase/kinase superfamily that includes heat-shock protein-90 (Hsp 90) and that share a unique ATP-binding pocket located in the C-terminus of PDKs. Conformational changes in portions of the binding pocket are coupled to ATP hydrolysis and protein-protein interactions, properties exploited by Tso and coworkers (145) in converting an established Hsp 90 inhibitor into novel inhibitors specific to each PDK isoform. The lead compound of this series, 2[(2,4-dihydrophenyl) sulfonyl] isoidoline-4,6-diol, with a Ki of 0.18 μM for PDK2, improved glucose tolerance and decreased hepatic stenosis in diet-induced obese mice. Others have targeted the same ATP-binding pocket shared by PDK and Hsp90 with 4,5 diarylisoxazole derivates to inhibit tumor cell PDK and proliferation. The pan-isoform ATP competitive inhibitor of PDK VER-246608 has little apparent activity against Hsp 90 but does exhibit modest in vitro antiproliferative activity in various cancer cell lines (146).

Finally, various PDK inhibitors, based on naturally occurring PDC substrates or cofactors (157,158) or on structurally novel pyrazole (98) or furan and thiophene carboxylate (159) compounds, have also been tested for initial inhibitory activity.

Is Chronic Suppression of PDK Good for You?

There are no known experiments of nature in which PDKs are absent or functionally inactive, save for one possible exception: an American strain of Doberman pinschers in which a 16 bp splice site deletion in the PDK4 gene reportedly is causally associated with the development of dilated cardiomyopathy (160,161). However, this finding could not be replicated in a larger cohort of Doberman pinschers of European origin (162). Nevertheless, fibroblasts from American Dobermans with dilated cardiomyopathy that were homozygous or heterozygous for the *PDK4* mutation reportedly did exhibit reduced mitochondrial oxidative capacity, compared with cells from dogs without the mutation (163). While intriguing, these controversial results do not provide convincing insight into the genetic or pharmacological health implications of long-term suppression of PDKs nor, by inference, of chronic activation of PDC. However, data from children with congenital deficiency of PDC or one or more complexes of the respiratory chain who have been exposed for several years to oral DCA provide no evidence of global or organ-specific toxicity, save for the possibility of asymptomatic reversible peripheral neuropathy (112,164).

Conclusions

PDK inhibitors represent an exciting field of research into the metabolic modulation of cancer, but several important questions remain to be adequately addressed. For example, given the cellular heterogeneity of tumors, it is almost certain that there exists important tissue variability in PDK isoform expression within a tumor. Thus, identifying a single isoform to "type" a cancer and use this information to "track" tumor progression or drug response may be problematic, particularly because tumor heterogeneity evolves during its natural progression and in response to therapy. In this regard, there have been no studies that have prospectively examined the relative expression or activity of PDK isoforms in cancer, although this may be crucial in understanding possible causes of tumor resistance to or escape from treatment. Therefore, preoperative or other pretreatment tissue PDK typing may be vital to guiding rational therapy with PDK inhibitors. Further research is also needed to better define the physiological relevance of post-translational modification of PDC apart from the canonical phosphorylation-dephosphorylation mechanism. In particular, such epigenetic

changes in the PDC/PDK axis could have far-reaching consequences in determining its role in multiple physiological and pathophysiological conditions. Lastly, and based on the data so far obtained only with DCA, it will be essential to determine the role of PDK inhibitors as adjuncts to standard radiotherapy and chemotherapeutics, in terms of both safety and efficacy. For example, it may be possible that such combination therapy can reduce patient exposure to standard treatments, thereby mitigating their toxicities. On the other hand, it will be important to investigate whether new PDK inhibitors share DCA's potential to induce peripheral neuropathy and whether this adverse effect is additive or synergistic with standard chemotherapeutic agents that are also potentially neurotoxic. Consequently, the kinetics and biotransformation of these agents must be elucidated prior to clinical testing.

Despite these caveats, there remains much hope that a new generation of small molecule regulators of the PDC/PDK axis will advance both the understanding of the biological importance of this metabolic junction box and foster the treatment of an expanding array of diseases in which mitochondrial energetics is perturbed. However, recently synthesized PDK inhibitors are still years away from proving their clinical utility. Until then, their structurally humble predecessor, consisting of chlorinated vinegar, remains actively investigated for its therapeutic effects. Personalized, genetics-based dosing of DCA for chronic administration should widen this orphan drug's therapeutic window and continue to advance our understanding of how regulation of the PDC/PDK axis integrates human physiology and disease.

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Notes

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