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Review

Molecular Cancer Therapeutics

Metabolic Flexibility in Cancer: Targeting the Pyruvate Dehydrogenase Kinase:Pyruvate Dehydrogenase Axis

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Abstract

Cancer cells use alterations of normal metabolic processes to sustain proliferation indefinitely. Transcriptional and post-transcriptional control of the pyruvate dehydrogenase kinase (PDK) family is one way in which cancer cells alter normal pyruvate metabolism to fuel proliferation. PDKs can phosphorylate and inactivate the pyruvate dehydrogenase complex (PDHC), which blocks oxidative metabolism of pyruvate by the mitochondria. This process is thought to enhance cancer cell growth by promoting anabolic pathways. Inhibition of PDKs induces cell death through increased PDH activity and subsequent increases in ROS production. The use of PDK inhibitors has seen widespread success as a potential therapeutic in laboratory models of multiple cancers; however, gaps still exist in our understanding of the biology of PDK regulation and function, especially in the context of individual PDKs. Efforts are currently underway to generate PDK-specific inhibitors and delineate the roles of individual PDK isozymes in specific cancers. The goal of this review is to understand the regulation of the PDK isozyme family, their role in cancer proliferation, and how to target this pathway therapeutically to specifically and effectively reduce cancer growth.

Introduction

Alterations in metabolism are a noted hallmark of cancer (1). Meeting the needs of highly proliferative cells requires increased production rate of intermediates required for growth, energy in the form of ATP, and a constant proproliferative cellular signal. Otto Warburg proposed in 1927 the idea that cancers use glycolysis to generate ATP which allows for this proliferative advantage, later termed the Warburg effect. Although the Warburg effect has largely been confirmed, recent efforts in the field have begun to unravel the markedly more complex metabolic processes that link genetic mutation to alterations in metabolism (2, 3). It is understood that although many cancers are capable of producing ATP through glycolysis, most cancers have functionally active mitochondria that play a central role in both cancer metabolism and tumorigenesis (2, 4). Central to mitochondrial function is a family of enzymes called the pyruvate dehydrogenase kinases (PDK1–4) and their interaction with pyruvate dehydrogenase (PDH) and the PDH complex (PDHC; ref. 5). Alterations in expression of the PDK family isozymes have been noted in a diverse array of cancers, and the PDK:PDH interaction has been proposed as a potential therapeutic target, especially with regards to enhancing the efficacy of other treatments (5). In spite of this, debate exists over their role in cancer, especially regarding specific family members in certain cancers. Furthermore, there are an increasing number of studies that indicate sustained upregulation of PDKs that should favor the Warburg effect may actually *limit* tumorigenesis. Anchoring these data, some studies indicate inhibition of PDH, the target protein of PDK, may also paradoxically limit tumorigenesis. The purpose of this review is to understand the varied roles of PDKs in cancer, with an emphasis on understanding how the PDK:PDH interaction can alter tumorigenesis and chemoresistance.

Pyruvate Dehydrogenase Kinases: Function and Regulation

The PDK:PDH interaction

The PDK family of enzymes comprises four members (PDK1–PDK4) that are located in the mitochondrial matrix with approximately 70% homology between them (6). PDKs phosphorylate serine residues Ser293 (Site 1), Ser300 (Site 2), and Ser 232 (Site 3) on the E1α subunit of PDH which serves to inactivate the PDHC (7). This activity is counterregulated by two pyruvate dehydrogenase phosphatase (PDP) enzymes which dephosphorylate PDH and reactivate the complex (8). Notably, the role of PDPs is underexplored relative to the role of the PDKs in the regulation of PDHC (5). The primary understood role of the PDHC is oxidative metabolism of pyruvate into acetyl-CoA via decarboxylation. Acetyl-CoA generated by the PDHC enters the tricarboxylic acid cycle where it can undergo further metabolism, resulting in the eventual formation of ATP by the electron transport chain (Fig. 1). Inhibition of PDHC conserves pyruvate for recycling of NAD^+ by lactate dehydrogenase, anaplerosis by pyruvate carboxylase, and transamination by alanine aminotransferase which hypothetically enhances the capacity for proliferation. Through their capacity to phosphorylate and inactivate the

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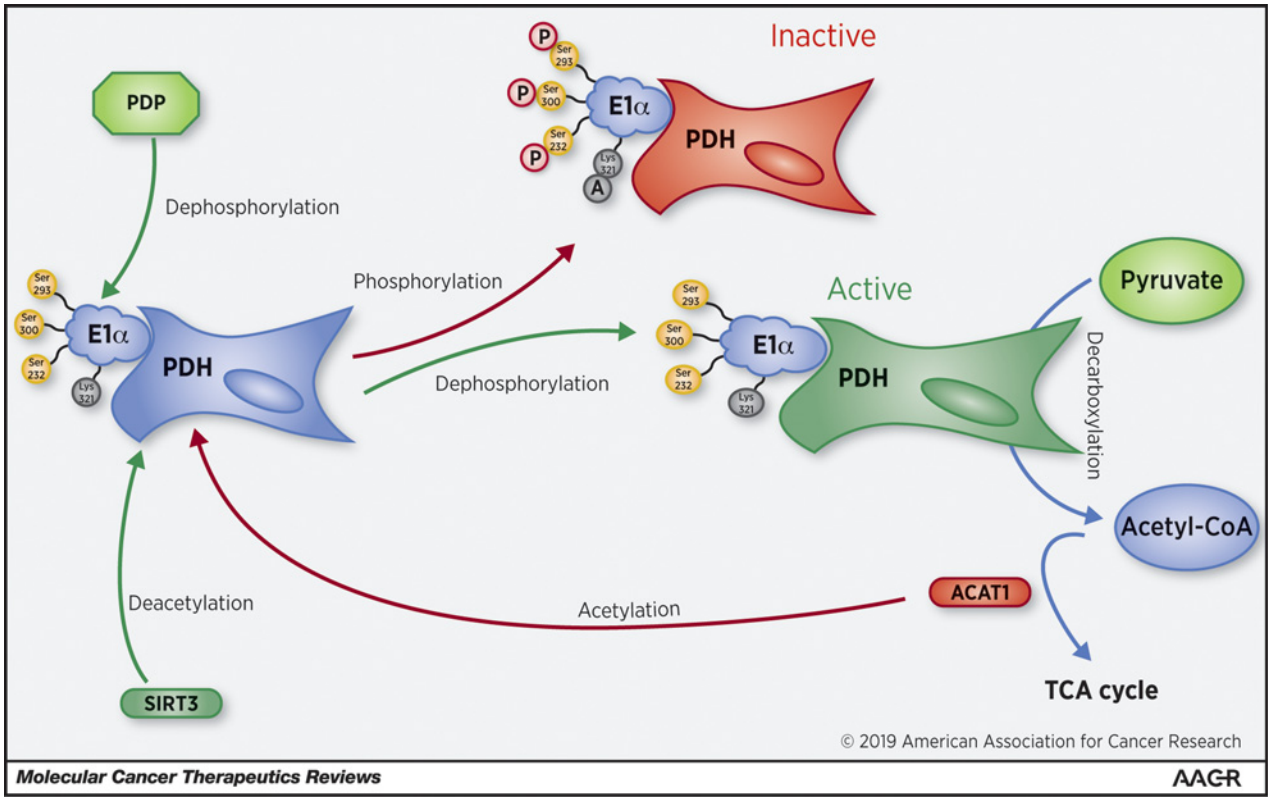

Figure 1.

Regulation of PDH by PDK. The E1α subunit of PDH undergoes phosphorylation at one of three serines or acetylation at lysine 321, which result in inactivation of the pyruvate dehydrogenase complex. The active form instead metabolizes pyruvate to acetyl-CoA for usage in the tricarboxylic acid cycle. Lys, lysine; Ser, serine; SIRT3, sirtuin 3.

PDHC, the PDK family exerts significant regulatory control over pyruvate metabolism, and thus cellular energy production and anabolic metabolism (5).

Inhibition of PDKs results in activation of the PDHC, when unopposed by PDPs, and results in increased mitochondrial respiration, reduced glycolysis, and increased ROS production and cell death in cancer cells (9). These data are further supported by the fact that acetylation, a second posttranslational modification of PDH, also appears to play an important role in regulation of PDH activity. Acetylation of the E1 alpha subunit of PDH on lysine 321 is catalyzed by acetyl-CoA acetyltransferase 1 (ACAT1), and deacetylation of this lysine is catalyzed by SiRT3 (10, 11). Acetylation inhibits PDH by recruiting PDK1 to the phosphorylation sites on PDH E1α (10, 11). Because PDK1 is the only PDK that phosphorylates site 3 (Ser232), phosphate occupancy of this site is a marker for the acetylated lysine mechanism. Oncogenic tyrosine kinases that activate ACAT1 promote acetylation and phosphorylation of PDH which reduces its activity and shifts metabolism further toward glycolysis (11). Blockade of PDH acetylation through inhibition of ACAT1 with a small-molecule inhibitor also enhances PDH activity and leads to reductions in cell growth, similar to the effect of PDK inhibition (11). It appears likely that upregulation or activation of ACAT1 and/or downregulation of SIRT3 may stimulate tumor growth by acetylation of PDH, recruitment of PDK1, phosphorylation of PDH, inhibition of pyruvate oxidation, and induction of the Warburg effect yielding a secondary means of PDH regulation that occurs in part through PDKs (12, 13).

The source of ROS after PDK inhibition is not well understood. The PDH complex is capable of generating ROS, and inhibition of the complex can reduce ROS generation, lending credence to the idea that activation of PDH may increase ROS production through PDHC itself (14–16). Surprisingly, some direct PDH inhibitors such as CPI-613, which have opposing effects to PDK inhibitors, also have chemotherapeutic effects in cancer cells, although this may involve other enzyme complexes such as α-ketoglutarate dehydrogenase *in vivo* (17). Moreover, some evidence exists that ROS may serve to further activate PDKs as suppression of mitochondrial ROS can block PDK activity in BRAF-mutant cells, although contradictory evidence indicates ROS may also inhibit PDK2 in normal cardiomyocytes (18, 19). ROS production also arises from complex I and complex III, more traditional sites of electron loss, and generation of superoxide. Metformin, a complex I inhibitor that also has anticancer activity, can synergistically induce cell death when combined with sodium dichloroacetate (DCA), and as such, significant interaction may be present that merits more direct investigation (20).

Regardless of its origin, dysregulation of the PDK:PDH interaction results in altered mitochondrial respiration and metabolism as well as increased ROS formation that yield far-reaching effects on cellular growth, cell signaling, oxidative stress, and

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cellular metabolism (5). In cancer, this commonly leads to reductions in proliferation, or cell death, and is a major proposed mechanism through which PDK inhibition can limit tumor growth. As such, cancer cells seem to be acutely sensitive to changes in the PDK:PDH axis, and thus limiting the metabolic flexibility augmented by alterations in the PDK:PDH axis may be a highly effective therapeutic target. Work is ongoing in this area in an attempt to better define downstream metabolic processes affected by this pathway.

Transcriptional regulation of PDKs

Genetic upregulation of PDK genes has been cited repeatedly as a potential mechanism through which transcription factors and regulatory factors such as miRNAs can control tumorigenesis (21–23). PDKs are widely expressed in a variety of tissues with evidence for tissue specificity (24, 25). Notably though, PDKs are also differentially regulated in cancer such that expression profiles in tumor are substantially different than those in the associated normal tissue (22–24, 26). Analysis of The Cancer Genome Atlas notes that many PDKs are differentially regulated in multiple cancers. Simultaneously, PDK activity is also regulated by covalent modification, allosteric effectors, and the relative activities of the PDPs. Therefore, understanding PDHC activity under cellular conditions, which is rarely measured relative to normal tissue, remains critically important regardless of the relative transcriptional changes in PDKs or PDPs. Nevertheless, PDKs are regulated by multiple transcriptions factors such as the hypoxia-inducible factor-1α (HIF1α) transcription factor (23, 27). Although HIF1α is normally broken down by prolyl hydroxylases, hypoxia stabilizes HIF1α and results in translocation to the nucleus where it can upregulate a multitude of genes involved in glycolysis, including PDK isozymes (28). This is likely a normal physiologic response to hypoxia, as the lack of oxygen would require ATP production through nonoxidative means. Many tumors take advantage of this response under both hypoxic and normoxic conditions though (28). Multiple cancer types have increased HIF1α activity due to overgrowth of the blood supply and lack of oxygen, in combination with genetic mutations in pathways such as Akt and mTOR that can stabilize and activate HIF1α during normoxia (18, 29). Upregulation of PDKs by HIF1α inactivates PDH, which conserves pyruvate for reduction to lactate by lactate dehydrogenase, resulting in recycling of NAD^+ and the production of ATP in the cytosol in what is normally an anaerobic reaction (2, 27). This occurs even in the presence of oxygen, yielding multiple metabolic pathways capable of generating ATP and metabolic intermediates (Warburg effect). As such, upregulation of PDKs in cancer can be drawn directly back to both transforming mutations and the hypoxic microenvironment in which tumors exist.

Similarly, peroxisome proliferator-activating receptor-α (PPAR-α) is thought to upregulate PDKs in normal tissue (30). PPAR-α is a key nutrient sensing nuclear hormone receptor that is activated under conditions of nutrient deprivation (31). Pharmacologic activation of PPAR-α activates PDK4 in a number of tissues (22). Fasting fails to upregulate PDK4 in the kidney in PPAR-α–deficient mice indicating PPAR-α is primarily responsible for the upregulation of PDK4 in fasting conditions (30). Although the primary role of PPAR-α is to initiate a transcriptional program responsible for ketogenesis and fatty acid oxidation, upregulation of PDKs may be a component of the response to nutrient deprivation. This falls in line with upregulation following hypoxia as a cellular response to reduced ATP availability during either starvation or oxygen deprivation. As such, PDK's indirect targeting of PDKs through suppression or inhibition of activating transcription factors like the PPARs or HIF1A or potentially activating posttranslational modifications may be an alternative means for targeting this pathway.

In contrast, some PDKs undergo downregulation in cancer. PDK4, which is usually expressed to a high degree in the liver, undergoes significant downregulation hepatocellular carcinoma (32, 33). Similarly, PDK4 is suppressed in lung cancer, which may be regulated by miR-182 (21). Some of these studies have suggested that PDK4 may have alternative suppressor functions, although this is still under investigation (21, 33).

Many cancer cell lines have substantial upregulation of PDK isozymes resulting in increased usage of glycolysis (23, 26, 34, 35). Increasingly, it has been noted that mitochondria are functional in cancer, and thus upregulation of PDKs and reduction in PDH activity should not be confused with nonfunctional mitochondria, which likely only occurs during complete anoxia where oxygen is absent (4). Suppression of the mitochondrial adenine nucleotide translocator sets a lower cytosolic ATP/ADP ratio and an abnormally higher cytosolic-free ADP concentration that allows simultaneous ATP synthesis by both glycolysis and oxidative phosphorylation, the latter by a nonelectrogenic mitochondrial ATP/ADP exchange (36). In other words, inhibition of glycolysis by mitochondrial respiration, that is, the Pasteur effect, is less effective in cancer cells because the mitochondrial ATP/ADP exchange is not driven by mitochondrial membrane potential. This allows for a dual energy/metabolism source and gives cancer cells a bipotent source for energy production and metabolite generation and likely contributes to the indefinite proliferation potential of cancer cells.

In summation, transcriptional control of PDK expression appears to be a key regulator of a system designed to give all cells metabolic flexibility during periods of stress. Cancer cells hijack this flexibility to sustain their infinite proliferative potential through both flexible energy generation and altered metabolic pathways that allow them to generate necessary metabolites through multiple means (Fig. 2). Inhibition of this flexibility through a variety of means is under examination as a mechanism for blocking proliferation or directly killing cancer cells.

PDKs in Cancer: Current Opportunities and Challenges

PDK inhibition as a therapeutic target

The initial observation that the pan-PDK inhibitor DCA effectively reduced proliferation of cancer cells through induction of ROS and subsequent apoptosis has led to a surge in studies on the use of PDK inhibitors (9). PDK inhibition with DCA has been tested *in vitro* and in animal models of kidney, bladder, head and neck, breast, and more (9, 26, 34, 37). The overwhelming majority of these papers have demonstrated reductions in phospho-PDH: PDH ratios, increased PDH activity, increased ROS production, and subsequent cell death after PDK inhibition/PDH activation (9, 26, 27, 34, 37). Similarly, a majority of these same studies report reduced glycolysis, reduced cytoplasmic lactate and pyruvate levels, and increased oxygen consumption, all of which are consistent with increased usage of pyruvate by the mitochondria (9, 34, 37). PDK inhibition at pharmacologically achievable

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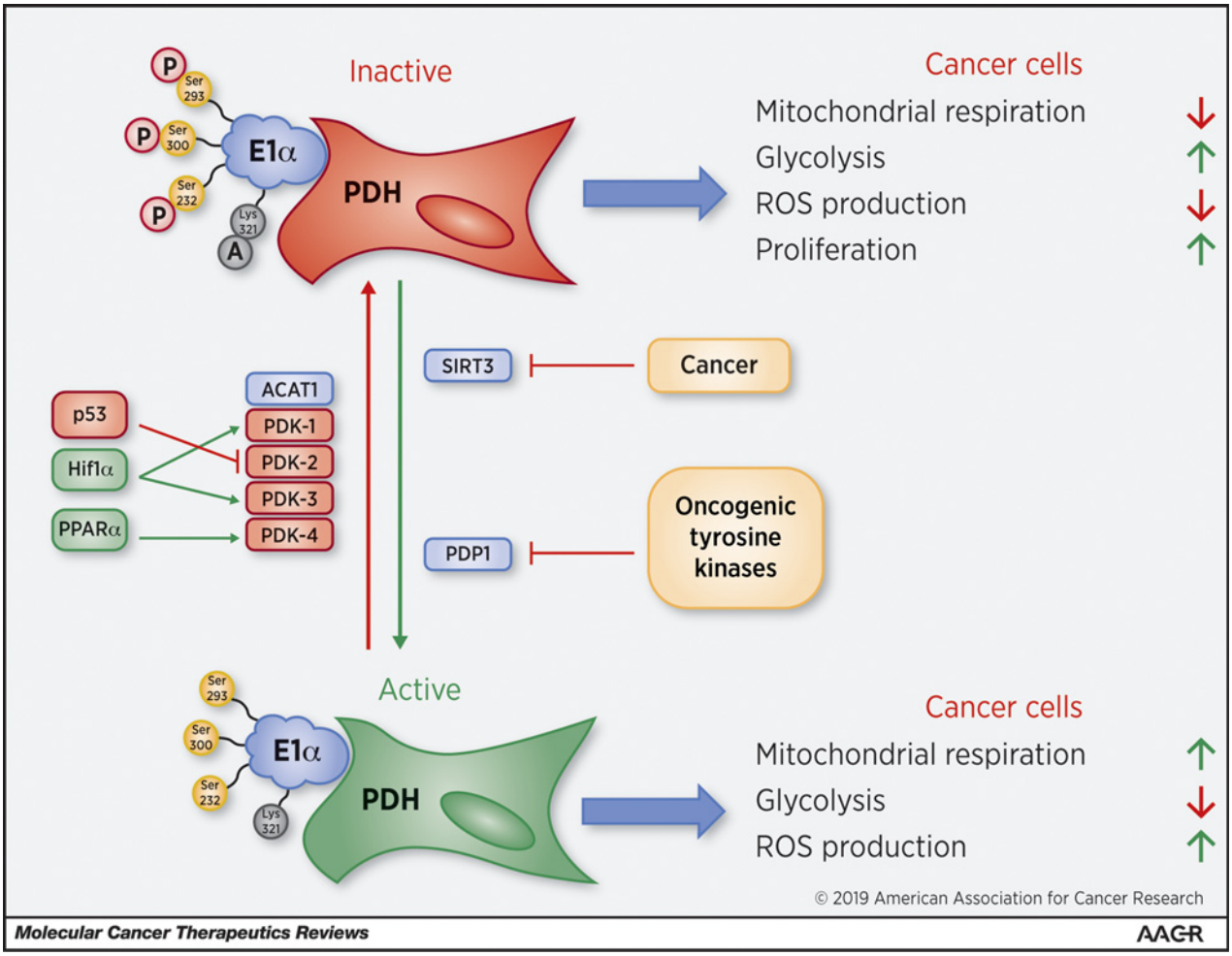

Figure 2.

Regulation of PDK. A number of transcription factors and other proteins regulate PDKs both positively and negatively. PDPs are also regulated by oncogenic tyrosine kinases, and counterregulate the PDK family. Ultimately, many of these regulatory signals converge on acetylation or phosphorylation of PDH. Lys, lysine; Ser, serine; SIRT3, sirtuin 3.

dosing has been shown to synergistically enhance the effects of other chemotherapeutics such as cisplatin, 5-fluorouracil, and doxorubicin, potentially giving it a role as a therapeutic adjuvant in multiple cancers (38, 39). These data all support the idea that removing the glycolytic energy source and forcing cancer cells to use their mitochondria both induces baseline ROS that can be toxic, as well as sensitizes cells to other cell death signals that act on the mitochondria.

Unfortunately, DCA requires exceptionally high concentrations (mmol/L or greater) because cancer cells silence expression of solute carrier protein 5A8, a sodium-linked, electrogenic monocarboxylate transporter responsible for DCA uptake in normal cells, and DCA also binds weakly to its target PDKs (40, 41). Furthermore, long-term therapy with DCA is problematic due to liver damage caused by inhibition of glutathione transferase Z and peripheral neuropathy perhaps due to oxidative nerve damage (42). Clinical trials testing the efficacy of DCA have been limited, but have reported data on optimized doses, although limited information on efficacy is available and one study reported the potential for severe toxicity, although this may not have been directly related to DCA (43, 44). Furthermore, a number of studies have used DCA to inhibit PDKs broadly, but have interpreted this with a more narrow focus on specific PDK isozymes. Although the efficacy of DCA and other PDK inhibitors in laboratory models of cancer is largely unquestioned, the role of individual PDKs and the mechanisms behind how PDKs enhance chemotherapy or induce cell death and the more complex pathways that dictate the regulation of individual PDKs in specific cancer remain areas of intense interest in the field. Moreover, given the nature of the enzyme family, it remains poorly understood if broad inhibition of all PDKs is required to initiate a response or if specific inhibition of single PDK isozymes with small molecules can yield the same effect with reduced toxicity in other tissues. Efforts are underway to generate PDK isotype-specific inhibitors to determine if pharmacologic intervention is possible. We will evaluate the contributions of each of the four specific PDK isozymes to specific cancers and their potential as therapeutic targets.

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PDK1. PDK1 (not to be confused with 3-phosphoinositide–dependent kinase-1, sometimes referred to as PDK1) has been extensively examined in cancer. PDK1 is often linked to either hypoxic or normoxic HIF1α expression which is a direct PDK1 regulator (13, 27). Hypoxia may also regulate PDK1 through mitochondrial accumulation of Akt2 and subsequent phosphorylation of PDK1 on the Thr346, representing a novel posttranslational modification that controls PDK activity independent of transcriptional control (45). HIF1α deficiency dramatically decreases cell growth under hypoxic conditions, which can be rescued almost entirely by forced expression of PDK1 (13). One of the initial studies on PDKs in cancer indicated normoxic stabilization of HIF1α enhances PDK1 activity in head and neck squamous cancer cells (HNSCC), and knockdown of PDK1 reverts this phenotype while also decreasing invasiveness, tumor growth, and reversing the Warburg effect (27). Similar data have been obtained in other cancers such as clear-cell renal carcinoma, breast cancer, and more (37, 46). PDK1 expression is also associated with poor prognosis in HNSCC, esophageal cancers, and more, further implicating it as a potential therapeutic target (47, 48).

PDK1 may also be involved in metastasis. Breast cancer cells that were metastatic to the liver were found to have increased HIF activity as well as increased PDK1 expression (35). PDK1 knockdown did not affect normoxic or hypoxic oxygen consumption rate or extracellular acidification rate, but did affect phosphorylation of PDH and capacity for metastasis (35). This same study indicated that in contrast to more recent data, PDK1 knockdown did not affect primary tumor growth of breast cancer cells (35, 46). This is in contrast to previous data indicating pan-specific inhibition of PDKs with DCA-induced cell death and limited tumor growth (9).

The recent advent of a PDK1-specific inhibitor that covalently binds and blocks PDK1 function indicates that specific inhibition of PDK1 can effectively limit A549 lung cancer cell and KELLY neuroblastoma cell growth *in vitro* and *in vivo* in the absence of PDK2-4 (49). Lead candidates from this class of compounds show considerable selectivity over other PDKs, with up to 40-fold differences in binding preference (49). This may be a means for significantly reducing toxicity as compared with pan-PDK inhibitors such as DCA, given the tissue-specific differences in expression of PDKs (24, 49). Other novel inhibitors based on the dichloroacetophone structure have also demonstrated high potency with relatively specific profiles for PDK1 inhibition over other PDKs (50, 51). Work in this area may provide novel inhibitors as well as methodology aimed at the generation of isozyme-specific inhibitors.

PDK2. PDK2 is widely expressed throughout a number of tissues and is also implicated in a number of cancer studies (24). PDK2 is the only PDK enzyme thus far that has been confirmed as a p53 target and may be a mechanism through which WT p53 controls metabolism (52). PDK2 may also be more difficult to target than some of the other PDK isozymes. PDK2$^{-/-}$ mice have demonstrated that PDK2 is a potent kinase, is essential to normal function, and protects against metabolic disturbances such as hepatic steatosis (53, 54). Importantly, these same studies also served as an initial demonstration of the ability of other PDK isozymes to compensate for loss of a specific family member; PDK2$^{-/-}$ mice have reduced levels of PDHE1α phosphorylation despite the PDK2 loss as there is overcompensation through compensatory upregulation of PDK1 (53).

In cancer, PDK2 has not been studied as extensively as PDK1 or PDK4; however, critical studies looking at PDK2 have elucidated important aspects about the PDK:PDH interaction. PDK2 knockdown in A549 lung cancer cells, or treatment with DCA, blocked HIF1α activity and reduced HIF1α protein levels (55). DCA did not further inhibit HIF activity nor did it have a further effect on PDH in the presence of PDK2 inhibition, indicating PDK2 is likely the primary agent responsible for PDH phosphorylation in non–small cell lung cancer (55). Importantly, this indicates that altering the PDK:PDH interaction may reflexively normalize overactivation of HIF1α in normoxic conditions, yielding a feedback loop that deprives cancer cells of their addiction to HIF1α upregulation and glycolysis (55). This effect has also been demonstrated in clear-cell renal carcinoma cells using DCA or knockdown of PDK1, indicating the effect is likely not limited to PDK2, but rather PDK2 mediates this effect in certain tissues (37).

Other studies using DCA to target PDKs other than PDK1 in HNSCC have attributed some of the effect of DCA-based inhibition to PDK2 (27, 34). Treatment of cisplatin-resistant HNSCC cells with DCA sensitized cells to cisplatin in a manner that was partially dependent on expression of PDHE1α and induced cell death (34). Notably though, PDK2 knockdown alone was not sufficient to reduce phosphorylation of PDHE1α, despite the fact it was overexpressed, although DCA reduced phosphorylation effectively, and perhaps more importantly, PDHE1α knockdown only reduced the effect of DCA by approximately 50% leaving open the potential for significant off-target effects of DCA (34). This is in contrast to previous data indicating PDK2 knockdown dramatically reduced PDH phosphorylation in other cell lines (27).

PDK3. PDK3 is generally less studied than the other 3 isozyme family members. PDK3 has the highest binding affinity for the E2 domain of PDH and also has the highest activity, with the lowest sensitivity to feedback inhibition from high levels of pyruvate (56). PDK3 is also induced by HIF1α, and forced expression of PDK3 yields increases in glycolysis and drug resistance in multiple cancer cell lines (57). PDK3 is also a target of the histone lysine demethylase JMJD2A–E2F1 complex and, alongside PDK1, mediates KDM4A-induced tumorigenesis (58). Similarities in the transcriptional control of PDK1 and PDK3 may necessitate further studies attempting to differentiate their expression and effects in different tissues.

PDK4. PDK4 is primarily expressed in muscle tissue and liver, although it is also expressed in other epithelial cells such as bladder, where it is dysregulated during cancer (26, 59, 60). Multiple transcription factors including PPAR-α, HIF1α, PPAR-gamma, and farnesoid X receptor are found to upregulate PDK4 expression, and many are noted to be activated during tumorigenesis (59, 61). PDK4 has specifically been implicated as protumorigenic in multiple cancers including bladder, colon, and more (26, 62). Generally, the indicated mechanism is metabolic programming as in the case of other tumors previously discussed.

PDK4 inhibition has also been widely associated with drug resistance. PDK4 knockdown reduced growth rates, and PDK4 inhibition via DCA sensitizes bladder cancer cells to cisplatin (26). Similarly, PDK4 upregulation is TGFß dependent in colon cancer, and PDK4 knockdown or inhibition sensitizes

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colon cancer cells to 5-fluorouracil (38). Similarly, PDK4 inhibition in tamoxifen-resistant breast cancer cells sensitized the cells to tamoxifen/fulvestrant treatment, indicating that attacking metabolic vulnerabilities may increase sensitivity to nontraditional chemotherapeutics as well (63). The ability of PDK4 inhibition to sensitize cells to drugs may be dependent on the specific drug as well as the tumor type, and should likely be investigated on a case-by-case basis. Furthermore, the usage of PDK4 may be tied in part to the mutational profile of the tumor. Colon or lung cancer cells with mutation in KRAS, but not colon or lung cancer cells without KRAS mutation were highly sensitive to PDK4 RNA interference (64).

Other studies have proposed PDK4 expression may be advantageous to constraining tumor growth (21, 32, 33, 65). Activation of PPAR-gamma has been proposed as a therapeutic option in bladder cancer and reduces growth of NCI-H2347 lymphoblastoma cells (65, 66). PPAR-gamma agonism by pioglitazone stimulates PDK4 expression and reduces cancer cell outgrowth in a PDK4-dependent fashion, and ROS production by pioglitazone was found to require PDK4 expression (65). Decreased expression of FAM210b in ovarian cancer resulted in reduced PDK4 expression (67). Although this would normally be expected to constrain tumor growth, it was found that reduced PDK4 expression enhanced migration and invasiveness, activated the epithelial–mesenchymal transition, and increased metastasis rates through control of mitochondrial energy production (67). These data are surprising in light of the fact that metastasis suppressors such as KISS1 are linked to reversal of the Warburg effect and increased mitochondrial function, consistent with what reduced PDK4 expression should entail (68). Similarly, MiR-182 overexpression is inversely correlated with PDK4 expression in lung cancer (21). Either knockdown of PDK4 or overexpression of miR-182 stimulates lung tumorigenesis (21). Furthermore, epithelial defense against cancer, a phenomenon wherein normal epithelial cells promote extrusion and subsequent death of transformed cells, was found to be PDK4 dependent (69). Paradoxically, the increased energy and metabolite production provided by the Warburg effect may help normal epithelial cells constrain transformed tumor growth (69). In contrast to cancer cells, PDK4 deficiency in normal hepatocytes expedites proliferation and progression through the cell cycle, whereas PDK4 knockdown in hepatocellular carcinoma cells results in apoptosis (33, 70). These are essentially opposite pathways, and these data largely oppose data from other studies indicating PDK knockdown can reduce growth rates of cancer, so it is not well understood why PDK4 genetic depletion has such opposing effects in different tumors and in normal versus tumor cells. Mechanistically, multiple groups have noted that increasing PDH activity may yield increased ATP production due to the efficiency of oxidative respiration (21, 67, 69). ATP levels are not the only thing required for cellular proliferation, and modern hypotheses on the Warburg effect have noted that the generation of intermediates that can be diverted into pathways that yield metabolites required for growth enhances tumorigenesis during Warburg metabolism (2). It is currently poorly understood what the downstream effects of PDK4 overexpression or knockdown broadly on metabolism are in cancer cells. Future studies aimed at determining how PDK4 overexpression, knockdown, or inhibition affects mitochondrial metabolism more broadly may demonstrate why these opposing effects are present. Moreover, given the large variety of contrasting effects noted with different approaches aimed at targeting PDK4, it may be necessary to further explore new hypotheses about the function of PDK4, especially in regards to its potential role as a tumor suppressor.

PDPs in cancer. Although considerable research has attempted to understand the role of PDKs in cancer, less effort has gone into understanding their counterregulatory enzymes, the PDPs. PDP1 and PDP2 dephosphorylate Site 1, Site 2, or Site 3 on the E1α subunit of PDH to reactivate the enzyme complex. PDP can undergo transcriptional regulation, although the mechanisms are not well understood in cancer, and this does not appear to be a widely observed and robust mechanism for regulation (71). Posttranslational tyrosine phosphorylation at Tyr-381 of PDP1 can control acetylation of both PDP and PDH and thus regulate activity (11). Moreover, phosphorylation at Tyr-94 directly inhibits PDP activity by blocking binding at the L2 domain of the E2 subunit and thus functionally blocking the interaction with PDH (72). These posttranslational modifications (PTM) may be direct means through which PDP activity is regulated in lieu of major transcriptional regulation. Importantly, the specific role of these PTMs in cancer is poorly understood, but may be a means for understanding regulation of PDKs and PDH's activity in the absence of obvious changes in PDK expression or activity. Moreover, indirect targeting of these factors may be a means for controlling cancer proliferation.

PDP is overexpressed in prostate cancer and is associated with increased PDH activity and increased mitochondrial oxygen consumption (73). Knockout of PDH in this model is protective against tumorigenesis (73). This also stands in stark contrast to the many papers that report that PDK inhibition and PDH enhancement reduce tumor growth, as this is essentially an opposing approach. Notably, the authors have proposed a novel role for nuclear expression of the PDH complex, although this has yet to be confirmed in other tissues. As such, there is increasing evidence that PDKs, PDPs, and the PDH complex all may be targetable therapeutically depending on individual cancers.

Therapeutic inhibition of the PDK:PDH axis: targeting metabolic flexibility

Cancer growth in different tissues has been blocked with use of multiple PDK inhibitors. Although DCA is a proven PDK inhibitor with activity in a number of tissues, clinical trials have thus far been disappointing (43, 44). A number of recent inhibitors with better specificity or more potency have recently been developed, although the majority of them still have IC_{50} values in the mmol/L range. A few specific inhibitors have been developed with μmol/L IC_{50} values, and improvements on both groups are continuing (49, 50).

In spite of the established effect of PDK inhibition, an increasing number of studies are now demonstrating that the larger picture may prove more complicated. Given that opposing approaches (PDK overexpression vs. PDK knockdown) have both shown to repress cancer growth, a tempting hypothesis is that pyruvate flux and metabolism is a highly controlled and delicate process in cancer cells. Lack of control of pyruvate metabolism results in overproduction of ROS, whereas complete suppression of metabolic flux limits the production of acetyl-CoA and other metabolic requisites needed by cancer cells. Although we have focused largely on glycolysis and respiration, disruptions in pyruvate flux interrupt not only glycolysis and mitochondrial

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respiration, but also anaplerosis, lipogenesis, and ß-oxidation and likely other metabolic pathways (54, 74). Moreover, metabolism in T cells is increasingly becoming an area of interest in cancer, as T-cell expansion plays a role in the immune response to cancer (75). PDK/PDH inhibition in orthotopic models of cancer needs to be examined in order to determine if adverse effects on immune regulation occur during PDK inhibition.

Cancer cells are known to develop "addiction" to specific pathways in order to continue perpetual proliferation. Many of the studies currently undertaken have been done in different cell lines, different tissues, and with a variety of different driving mutations. It is entirely possible that different tissues under different mutational profiles demonstrate fundamentally different metabolic addictions. Targeting these more specifically in the age of precision medicine should remain a goal of the field, as we may be able to identify how specific inhibitors affect specific cancers based on metabolomic, genomic, or transcriptomic profiles. Finally, as molecular subtyping of tumors becomes more prevalent, it will be imperative to determine which subtypes are sensitive to PDK inhibition, and whether or not this provides therapeutic access to tumors not typically associated with chemotherapy.

Therapeutic inhibition of the PDK:PDH axis: combination therapy

Many papers have reported either DCA or PDK inhibition improves cisplatin-based therapy (5, 26, 34, 43). This is thought to occur through enhanced mitochondrial ROS production and enhanced susceptibility to mitochondrial-induced cell death, although these mechanisms are poorly described (9, 34, 76). It has been proposed that the reason DCA is toxic to cancer cells is their reliance on aerobic glycolysis for ATP production (or alternately, because of their metabolic dependency on aerobic glycolysis for proliferation) and thus DCA is less toxic to normal cells due to metabolic homeostasis (76). Another difference may be that DCA increases ROS in cancer cells beyond what can be safely handled by antioxidant mechanisms. Further overloading of cancer cells with ROS may contribute to the toxic synergism between DCA and chemotherapeutic agents. DCA in particular, likely because of its pan-inhibition of PDKs, synergizes with a number of other compounds as previously noted, including metabolic modulators such as metformin and growth factor receptor inhibitors such as erlotinib (20, 26, 34, 77). Notably, metformin usage is associated with increased recurrence/progression-free survival, and reduced cancer-specific mortality and thus PDK inhibitor combination therapy may be useful for bladder cancer prevention if toxicity can be mitigated (78). DCA is tolerated in patients undergoing treatment for lactic acidosis, but notably the concentration given is below that used for cancer studies (79). Given the consistency in DCA/PDK inhibition improving other therapies, incorporation of PDKs into current therapeutics seems like a highly likely scenario for improving therapy.

Conclusions

The PDK:PDH interaction remains an area of intense therapeutic interest. Refined studies are needed to specifically address how inhibition or activation of this interaction results in tumor growth inhibition. Moreover, novel inhibitors with superior potency and pharmacokinetics are needed to advance the field forward therapeutically.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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