We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

The Pyruvate Dehydrogenase Complex in Cancer: Implications for the Transformed State and Cancer Chemotherapy

Paul M. Bingham and Zuzana Zachar

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/48582>

1. Introduction

The problem of finding effective chemotherapies for advanced cancer remains largely unsolved. We review here the role of a specific class of central metabolic dehydrogenases whose regulatory properties are remodeled significantly in cancer cells. These remodeled properties may provide an attractive set of targets for the development of new chemotherapeutic drugs. The design features of the first agents to be developed attacking these new targets [1,2] may illustrate ways to exploit this potentially valuable therapeutic opportunity.

2. The central role of the pyruvate dehydrogenase (PDH) and alpha-ketoglutarate (KGDH) complexes in cell metabolism

The evolution of the high-energy, oxygen-dependent metabolism of eukaryotes [4,5] has produced mitochondrial metabolic pathways whose control is centrally focused on a series of dehydrogenases. Two of these pivotal dehydrogenases, the pyruvate dehydrogenase (PDH) and alpha-ketoglutarate or 2-oxoglutarate (KGDH) complex will be our central focus here. These dehydrogenases control the entry of carbon into the TCA cycle from two major sources, carbohydrate and gluconeogenic amino acids (pyruvate; PDH) and glutamine (KGDH), the most abundant serum amino acid and a central carbon source for normal and pathological tissues (Figure 1). The TCA cycle, in turn, is almost exclusively responsible for the capture of reducing potential from nutrients for the purpose of driving the oxygen-dependent mitochondrial electron transport system for ATP production [6].

In the solid, three dimensional structure of animal bodies the availability of nutrients and molecular oxygen is locally contingent and dynamically variable. Thus, continuous real-time

control of PDH and KGDH activities is essential to proper function. Moreover, in various pathological contexts these regulatory processes are substantially altered in ways that are essential to that pathology and this may reflect targets for therapeutic intervention (see sections 5 and 8 below for detailed references).

Figure 1. The pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (KGDH) complexes govern the entry of carbohydrate- and glutamine-derived carbon, respectively, into the TCA cycle. Glucose and glutamine are two major carbon sources feeding many mammalian cells, including tumor cells. These molecules are metabolized to support ATP generation, through cytosolic glycolysis and oxygen-dependent mitochondrial metabolism in which most reducing potential is derived from the TCA cycle. As well, both carbon and reducing potential from glucose and glutamine are delivered to anabolic, biosynthetic functions. Among these are provision of citrate and NADPH for lipid biosynthesis as illustrated and delivery of carbon skeletons from glycolysis and the TCA cycle for amino acid biosynthesis (not illustrated). The entry of glucose derived carbon (either from dietary glucose or from amino acids used for gluconeogenesis) is controlled by the PDH complex and the entry of glutamine derived carbon is controlled by the KGCH complex. Figure redrawn from several sources, including [3].

PDH decarboxylates pyruvate, releasing carbon dioxide, capturing reducing potential and transferring the two-carbon acyl unit (acetate) to Co-enzyme A (CoA) (Figure 2). Structurally homologous KGDH catalyzes the analogous reaction, decarboxylating alpha-ketoglutarate and generating succinyl-CoA. Each of these reactions is highly exergonic and also generates rapidly dispersed or consumed products (carbon dioxide and acyl-CoA). Thus, the initial decarboxylation reaction in each case is effectively irreversible, reflecting a forward commitment step.

The catalytic details of PDH and KGDH are as follows (Figures 2 and 3). Each complex contains enzymatic activities conventionally referred to as E1, E2, and E3. E1 catalyzes oxidative decarboxylation (of pyruvate or alpha-ketoglutarate, respectively) using a thiamine pyrophosphate co-enzyme. The activated acyl unit created by this process is transferred to sulfhydryl groups of a lipoic acid (lipoate) residue of an E2 subunit. E2 lipoate is in the form of lipoamide, in which the carboxylic acid moiety of lipoate is joined in amide linkage to epsilon amino group of an E2 active site lysine. The two sulfurs on the lipoate moiety (Figures 3) exist in a disulfide configuration which is reduced and acylated (thioesters) to produce activated acetate or succinate residues. The E2 subunit further catalyzes the transfer of the lipoate-activated acyl residue to CoA leaving dihydro-lipoamide. Finally, the E3 subunit catalyzes the transfer of the reducing potential in the dihydro-lipoamide residues of E2 to NAD⁺ (through enzyme disulfide to FAD to NAD steps) [7,8] to generate NADH for transfer of the reducing potential to the electron transport complex and regenerating oxidized lipoate (Figure 2).

Both PDH and KGDH are very large complexes containing multiple copies of each of these three key enzymatic activities (Figure 3). For example, mammalian PDH consists of 60 copies of the E2 subunit (48 of E2 itself and 12 of the E2 homolog called the E3 binding protein, E3BP; Figure 3) [9].

This organization allows efficient channeling of the products of the intermediate reactions [10]. Flux through these complexes is subject to extensive regulation as expected from their central role in governing carbon flow in the face of variable supply and demand. The end products of PDH and KGDH activity, NADH and acyl-CoA, inhibit complex activity directly, apparently by binding to the E2 and E3 active sites, respectively [11,12]. However, PDH is much more extensively regulated through its associated kinases and phosphatases [13].

The four PDKs that regulate the mammalian PDH complex are a novel class of kinases apparently unrelated to the large families of serine-threonine and tyrosine kinases so prominent in other mammalian regulatory processes as reviewed in [7,8]. These kinases are named on the basis of their order of discovery, PDKs1-4. Though the PDKs represent a related family of kinases, the sequence divergence between the four different isoforms (61-69%) is consistent with their significantly divergent regulatory behavior [14,15]. Moreover, PDK isoform function is apparently ancient and essential as the corresponding isoforms in rodents and humans are at least 94% conserved as reviewed in [15].

Figure 2. The catalytic cycle of the pyruvate dehydrogenase (PDH) complex. This cycle begins with the oxidative decarboxylation of pyruvate catalyzed by the E1 subunit, generating carbon dioxide and an activated two carbon unit (hydroethyl thiamine pyrophosphate, HE-TPP) as illustrated at the 10 o'clock position. E1 then catalyzes the transfer of this two carbon unit to one of the lipoate residues of the E2 subunits, creating acyl-lipoamide as illustrated at the 12 o'clock position. The E2 catalytic activity transfers this two carbon unit to CoA, creating acetyl-CoA and dihydro-lipoamide as illustrated at the 4 o'clock position. Finally, E3 catalyzes the transfer of the dihydro-lipoamide to NADH (through protein sulfhydryl and FAD intermediates) to recreate the oxidize form, lipoamide, as illustrated at the 8 o'clock position. Both the E2 catalyzed transfer of the lipoamide acyl group to CoA and the E3 catalyzed transfer of dihydro-lipoamide reducing potential to NADH are highly reversible reactions. Figure redrawn from several sources, including [8].

PDH E2 lipoyl domains bind and control PDKs

Figure 3. Functional organization of the pyruvate dehydrogenase (PDH) complex in mammals. This complex catalyzes the oxidative decarboxylation of pyruvate to yield acetyl-CoA to feed carbohydrate carbon into the TCA cycle and to capture reducing potential in NADH for transfer to the electron transport complex (ETC) to support ATP synthesis during step down transfer to molecular oxygen (Figure 2). The mammalian PDH complex is built around a core consisting of the inner domains of ca. 48 copies of the catalytically active E2 protein and 12 copies of the catalytically inactive E2 homolog, E3BP. The E1 subunit binds to E2 and the E3 subunit binds to E3BP. Both E2 and E3BP contain lipoate residues joined in amide linkage to the epsilon amino groups of specific lysine residues in the lipoyl domains (L1-L3). The lipoamide residues on the two lipoyl domains of E2 and the single domain of E3BP are acylated, reduced and re-oxidized during the PDH catalytic cycle (Figure 2). The zig-zag lines indicate highly flexible proline-rich domains allowing the E2 subunits high mobility throughout the complex and, perhaps, beyond. The regulatory kinases (PDKs) control PDH activity by phosphorylating (inactivating) the E1 subunit of the complex. Their activity is antagonized by two phosphatases (PDPs). The status of E2 lipoate residues strongly modulates the activity of the PDKs with acylation/reduction stimulating PDK activity. Figure redrawn from several sources, including [7]. The PDKs normally function as dimers [see, for example 16,17]. Of particular potential importance is the observation that at least two of the PDK isoforms (PDK1 & 2) readily form heterodimers [18]. This raises the possibility that there might be as many as 10 different PDK isoforms (four homodimers and six heterodimers). In view of the substantial changes in PDK levels associated with malignancy (below), this potential complexity may be very important and is worthy of more investigation.

The PDKs regulate PDH complex activity by responding to diverse allosteric modulators. High ratios of acetyl-CoA to CoASH and NADH to NAD⁺ represent signals of saturation of mitochondrial demand. Elevations of these ratios are strong allosteric activators of PDKs,

shutting down PDH activity in response to demand saturation [19, 20, 21]. Though saturation of mitochondrial demand is most often produced in response to high fat or carbohydrate intake in healthy animals, as reviewed in [8], the altered metabolism of tumor cells also creates a very new metabolic environment wherein these regulatory processes may be entrained for different purposes (below).

PDKs are also subject to allosteric regulation by pyruvate [22]. Elevated pyruvate levels interact synergistically with ADP to inhibit PDK activity (activating PDH). This PDK inhibition apparently results from the binding of pyruvate to PDK-ADP inhibiting exchange for ATP and, thus, phosphorylation. Dichloroacetate (DCA) is a pyruvate analog and acts very similarly to pyruvate in this allosteric modulation of PDK activity [22]. DCA was subsequently investigated as an anticancer drug (below) on the basis of this observation.

The final relevant feature of PDK regulation is the response of these regulatory kinases to the lipoyl domains of the E2 subunits of PDH [7, 8, 23]. PDK1-3 bind strongly to the L2 lipoyl domain of E2 and more weakly to the E2 L1 lipoyl domain (Figure 3). PDK4 binds to the L1 E2 domain and to the sole lipoyl domain of E3BP (Figure 3). This lipoyl domain binding requires the lipoamide component of the domain. Moreover, the well characterized binding of PDK3 to the E2 L2 domain defines the binding site in detail including the amino acids interacting with the lipoamide residue [16]. These residues are highly conserved in PDK structure [17] indicating that this binding mode is likely universal to the PDKs. This binding of PDKs to E2 lipoyl domains juxtaposes them to their E1 target (associated with the E2 subunit binding domain; Figure 3), producing a large increase in the kinase reaction rate. PDK dimers apparently interact simultaneously with two lipoyl domains, producing very high binding affinity to the PDH complex and supporting hand-over-hand migration of the PDKs through the complex [24].

Most importantly for our purposes here, the reduction and acylation state of the E2 lipoates strongly modulates associated PDK activity [25, 26]. Specifically, acylation and reduction of lipoate enhances PDK2 activity [7,8, 27]. Moreover, acetyl-CoA and NADH can be used as substrates to run elements of the PDH reaction (above) in reverse, thereby creating reduced and/or acylated lipoate residues in response to elevation in the levels of acetyl-CoA and NADH in the mitochondrial matrix [25,26]. In other words, the lipoate residues of PDH represent a real-time census of these crucial features of the energy status of the mitochondrial matrix.

Several additional details of lipoate regulation of PDH and KGDH are also relevant for this discussion. The lipoates in PDH and KGDH comprise a dense cloud of highly mobile residues [28], potentially interacting with crucial regulatory functions. This condition results from several features of the structure of these complexes. PDH illustrates the crucial issues. Each of the ca. 48 E2 subunits contains 2 lipoate (lipoamide) residues and each of the 12 E3BP proteins contains 1 such residue, for a total of 108 lipoate residues in each complex. The regulatory kinases (PDKs-1-4) interact with these lipoate residues embedded in the lipoyl domains (Figure 3). Moreover, these lipoyl domains not only have the boom-like structure resulting from their connection to lysine epsilon amino groups in the E3 lipoyl

binding domains, but also each of these domains is separated by highly flexible (alanine/proline-rich) linker domain.

These details will be important to us below in considering the possible mechanisms of action to pursue in developing new drugs based on lipoate structure. Specifically, each PDK can potentially interact simultaneously with two lipoate residues, as well as rapidly migrate between residues. This creates an environment in which PDKs may effectively sample the lipoate cloud, regulating their activity in response to what is effectively a measurement of the aggregate cloud status, especially the acylation/redox status of lipoate as determined by both forward flux through the complex and by backward charging of lipoate from NADH and acetyl-CoA products (above).

Such a sampling procedure would yield especially robust assessment of enzyme saturation and, thus, allow PDK control of PDH to be more reliably connected to the global mitochondrial energy status. *In vitro* studies demonstrate that free lipoate can interact with the PDH (and KGDH) complexes [29]. Thus, we anticipate that exogenous lipoate analogs might be effective in modulating the activity of PDK bound to the PDH complex (below).

3. Clinical implications of the reconfiguration of metabolism in cancer cells

The hypothesis that metabolism may be altered in important ways in cancer is longstanding [30]. However, our understanding of cancer metabolism and its relationship to malignancy and clinical outcome has only recently begun to receive extensive attention. Though our knowledge about cancer metabolism remains quite incomplete, some important global insights have emerged.

First, fluxes of both glucose and glutamine, two major carbon sources, are substantially up-regulated in many tumors as reviewed in [31-36]. More specifically, mutational changes altering signaling through the Akt pathway enhance glucose uptake and glycolytic metabolism [37]. Likewise, alterations of Myc expression substantially up-regulate glutamine utilization [3, 38, 39].

Second, evidence from multiple perspectives supports the view that one of the major functions of these tumor-specific changes is the redirection of metabolism toward provision of increased levels of anabolic substrates. For example, large fractions of glucose carbon are diverted into the pentose phosphate pathway in support of nucleotide synthesis and into amino acid biosynthesis. Likewise, a large portion of glutamine carbon is siphoned off of the TCA cycle as citrate to support lipid biosynthesis (including fatty acids and sterols) (Figure 1). We can call this the *anabolic shift* in tumor cells.

The therapeutic implications of the alterations in cancer metabolism depend heavily on how unique these changes are to tumor cells. There is good evidence that some elements of the anabolic shift are not unique to tumors cells, but may also be shared with rapidly dividing normal cells. For example, activated lymphocytes show some of these same metabolic

properties [40]. However, there is also reason to suspect that other features of the cancer cell version of the anabolic shift will not be shared with most normal, rapidly dividing cells. In particular, the poor vascularization of solid tumors reflects an environment rather different than rapidly dividing normal cells typically experience [41, 42]. Tumor cells are likely to have additional metabolic adaptations to this extreme environment.

Of course, the existence of such metabolic adaptations to the extreme tumor microenvironment may still not be helpful therapeutically if these adaptations are idiosyncratic to each individual case of cancer. Fortunately, there is both empirical and theoretical reason to believe that these special adaptations will, in fact, be generally consistent and predictable, as we now discuss. It has long been recognized that solid tumors bear a striking resemblance to healing wounds [43-45]. Specifically, solid tumors resemble wounds that continue the early steps in wound healing, but never resolve as normal wound healing does. Thus, it is plausible to speculate that metabolic modifications in tumor cells usually or always involve the pathological, uncontrolled activation of regulatory pathways normally accommodating cells to the rigors of the wound environment. On this working hypothesis, tumor metabolism is expected to have features not shared with normal cells (except potentially in healing wounds) and to be potentially universal to most or all cancers.

The possibility that tumor metabolism might have unique features is particularly important therapeutically. The dramatic increase in our knowledge about the genetic changes in cancer and our experience with genetically targeted therapies has produced some sobering potential insights as illustrated by the following examples. Specifically, in many or even most cancers, the “driver” mutations may represent loss-of-function in tumor suppressors rather than gain-of-function mutations [46-48]. Moreover, the fraction of tumor-producing changes that are epigenetic may be high. All of these features mean that targeting specific oncogene-targeted products, such as small molecules like gleevec, may be viable in only a minority of tumors [49].

Finally, the levels of redundancy in the regulatory circuits mutated in cancers mean that targeting an individual gain-of-function driver, even when it is possible, may be incompletely successful and subject to evolved resistance.

The upshot of these genetic considerations is that chemotherapy might be better focused on *properties* of the transformed state rather than on the genetic drivers producing that state. Moreover, non-redundant, indispensable properties of the transformed state are expected to represent the most propitious of such targets. Cancer metabolism may represent an environment in which such desirable targets exist. Below we will develop the argument that the dehydrogenases at key control points in metabolism are especially attractive candidates for such targets.

4. Modification of PDH regulation is vital to malignant transformation

If cancer metabolism is to be a therapeutic target it is vital to identify likely targets for intervention. In this section we will review some of the evidence that the PDKs regulating

PDH activity are excellent potential candidates for such targets. Later we will return to the question of whether the other major dehydrogenase entry point for mitochondrial metabolism, KGDH, might also be an attractive therapeutic target.

PDKs have long been recognized as key regulators of PDH function (above). Moreover, in view of the central role of modification of carbohydrate metabolism in cancer (above), alteration of PDK regulation in malignancy is likely. Initially, analysis of clinical samples demonstrated that up-regulation of PDK levels was a frequent correlate of advanced malignancy [50] a pattern that has persisted in subsequent studies [51].

These and other observations stimulated systematic detailed study to great effect. Kim, et al., [52] and Papandreou, et al. [53] demonstrated that HIF-1, a global regulator of the hypoxic response, up-regulated PDK1, with the expected effect of down-regulating carbon flow through PDH. Moreover, PDK up-regulation correlated with increased resistance to programmed cell death, a hallmark of cancer as reviewed in [54]. In view of the consistent hypoxia in solid tumors as reviewed in [41, 42] and the frequent activation of HIF1 in tumors as reviewed in [55] these observations suggested that PDK up-regulation might be a central element of the cancer metabolic reconfiguration.

The next challenge was to ask if more direct evidence for the importance of PDK up-regulation in cancer metabolism could be found. Was PDK up-regulation required for malignancy or marginal, epiphenomenal? Several observations argue strongly that PDK up-regulation is essential to the malignant phenotype.

First, McFate, et al. [56] demonstrated that PDK1 up-regulation was essential for the malignant phenotype in head and neck squamous cell carcinoma cells (HNSC). Specifically, PDK1 expression contributed to induction of limited flux of carbon through mitochondrial metabolism and PKD1 over-expression further reduced this flux (as measured by glucose consumption and diversion of carbohydrate carbon to lactate formation). Confirming this relationship, PKD1 knockdown increased TCA processing of carbohydrate carbon. PDK1 knockdown reduced several indicators of malignant potential, including ability to resist cell death under hypoxia or to form colonies in soft agar in culture. Most importantly, PDK1 knockdown dramatically, reduced the rate of tumor growth in xenograft models.

Earlier work from this group indicated that accumulation of glycolytic intermediates might, itself, be sufficient to up-regulate HIF1 [57]. Thus, McFate [56] proposed a positive feedback loop central to the malignant state wherein PDK1 up-regulation by HIF1 enhanced production of glycolytic intermediates (by blocking pyruvate consumption) further up-regulating HIF1 and so on.

Second, Lu, et al. [58] found a similar role for HIF1-dependent up-regulation of PDK3 in malignant metabolic state. In this case also, reduction in mitochondrial carbohydrate metabolism with the corresponding up-regulation of oxygen-independent cytosolic glycolysis was driven by elevated PDK3 expression. Of particular importance here, the resistance of tumor cells to killing by chemotherapeutic agents paclitaxel and cisplatin was strongly improved by PDK3 up-regulation, while PDK3 knockdown sensitized to these

agents. Finally, Lu, et al. [58] observed that both PDK1 and PDK3 collaborated to produce this drug resistance.

Collectively, these results clearly indicate that PDK regulation of PDH activity is central to the malignant phenotype. Moreover, there is apparently no metabolic bypass of the requirement for properly managed mitochondrial metabolism. Mitochondria are not turned off in hypoxic tumor cells. Rather, they are redeployed to provide the limited carbon traffic they can sustain to the provision of anabolic intermediates (above). There appears to be no other way to provide some of these biosynthetic resources (citrate for lipid biosynthesis, for example). Further, mitochondria are central controllers of Ca^{++} signaling [see 59 and references therein] and of cell death control as reviewed in [60,61]. Thus, their continued properly controlled function is likely to be essential to cancer cell homeostasis and survival. These results and considerations are consistent with the hypothesis that this re-regulation of PDH cannot be dispensed with. Without this metabolic pattern cancer cells are likely to be unable to survive and prosper in the hypoxic environment crucial to malignant progression. Thus, PDK re-regulation is a candidate for the kind of essential, non-redundant therapeutic target needed if we are to have fundamentally new clinical options. We will argue below that this target can be therapeutically exploited in several different ways.

Before turning to assessment of how we might attack this potential target it is important to emphasize a particular implication of PDK re-regulation in cancer cells. Because of the possibility of PDK heterodimer formation (above), tumor-specific quantitative changes in individual PDK expression may be substantially amplified in their regulatory effects. For example, if PDK1 and PDK3 were both up-regulated 10 fold in the *in vivo* tumor environment, this could represent a 100-fold increase in the levels of the hypothetical PDK1/PDK3 heterodimer. Such very large effects might represent targets that are essentially qualitatively unique to tumor cells.

5. DCA, attempting to reverse tumor-specific PDK repression of PDH activity

Dichloroacetate (DCA) is a small molecule with a long history of clinical use for treatment of elevated serum lactate levels in some inherited metabolic conditions [62]. Moreover, DCA is well characterized as being a non-metabolizable pyruvate analog that can strongly inhibit PDK function [17,22, 63]. Thus, DCA is a candidate to reverse PDK repression of PDH important to cancer cell tumorigenicity (above).

These considerations influenced Bonnet [1] to investigate DCA's potential anti-cancer effects. These authors observed that DCA treatment could reverse the mitochondrial membrane hyper-polarization characteristic of tumor cells without noticeable effect on the lower level of polarization in normal cells. Further, DCA substantially increased glucose oxidation while decreasing fatty acid oxidation in A549 tumor cells [1]. These and other effects correlate with DCA inhibition of cancer cell growth and induction of a modest increase in the rate of cancer cell apoptosis. Finally and most importantly, DCA very

significantly inhibits tumor growth in mouse xenograft models. Specifically, DCA administered ad libitum in drinking water at 75mg/l produced both inhibition of xenograft tumor growth and modest, but significant reduction in tumor volume in larger tumors. Subsequent reports from other groups corroborate these preclinical findings [64, 65].

The clinical follow up on these preclinical results has been generally weak. This largely results from economic factors. DCA is a cheap, generic drug and no corporate actors have an incentive to develop the agent. To date clinical data consist of several anecdotal case reports [66,67] suggesting possible efficacy, including in a poorly differentiated metastatic carcinoma. In addition, in view of its low cost and wide availability, DCA is being prescribed off-label or self-administered. Anecdotal reports of efficacy from this use group can be found in patient/physician webshops (see, for example, www.thedcasite.com). It will be of considerable interest to see if sufficient evidence can be accumulated to assess the clinical potential of this agent.

An apparently common side effect of DCA use is peripheral neuropathy. Though this is generally limited and reversible, this may not always be the case. A patient report describes relatively severe encephalopathy probably induced by DCA doses within the range currently being experimented with by patients and their physicians [68]. These problems emphasize the importance for individual patients of using DCA only under professional medical supervision.

Finally, a series of small molecule PDK inhibitors have been developed, initially for the treatment of diabetes as reviewed in [8]. These compounds are far more potent than DCA on a mole basis and it will be of interest to see if they have significant anti-cancer activity. To date, no clinical trial data are available on any possible anti-cancer activities of these agents. Moreover, the design strategy for these compounds renders them relatively selective for individual PDK homodimers. We do not currently know which homo- or heterodimer(s) might be the most propitious anti-cancer target(s) for this class of agents.

6. Thioctoid lipoate analogs exaggerate cancer-specific, PDK-mediated repression of PDH to shut down tumor cell mitochondria and induce cell death

The PDH complex includes a dense cloud of lipoate residues making up the functional catalytic co-factor domain of the E2 activity (above). Acyl groups and reducing potential can be back-transferred to this lipoate cloud from the surrounding mitochondrial matrix pool as well as being generated by the conventional PDH forward reaction (above). Moreover, these acyl groups and reducing potential can apparently be rapidly shuttled between these residues before ultimately being transferred to CoA and NAD⁺[28]. Finally, the oxidation/acylation state of the elements of the lipoate cloud strongly effect the activity of the PDK regulatory kinases, with acylation/reduction stimulating PDK activity (above). Thus, each PDK homo- or heterodimer docked at any point on the PDH complex is apparently continuously exposed to the result of an ongoing, real-time poll of the

mitochondrial matrix energy status. Further, these PDKs are then able to act rapidly on all their E1 targets in the PDH complex in response to these polling results as a result of the combination of the highly flexible linkers surrounding the lipoyl domains to which they are bound (Figure 3) and their ability to move hand-over-hand through the complex.

In vitro studies demonstrate that free lipoate and lipoate-containing lipoyl domain fragments can interact productively with the intact PDH complex (above). This suggests that lipoate analogs might be able to perturb PDH regulation *in vivo*. Moreover, this perturbation might show strong selectivity for tumor cells in view of the substantial reconfiguration of PDK regulation in cancer (above). Based on the original PDH regulatory biochemistry and the presumption, subsequently confirmed (above), that PDH regulation was likely to be substantially altered in tumor cells, we began a systematic study of lipoate analogs as anti-cancer agents in the late 1990s (Patent # 6331559, filed 1999). The concept was that appropriately designed lipoate analogs might mimic the effects of specific intermediate states of lipoate (Figures 3 and 4) on PDK function and, thereby, misinform the PDH regulatory machinery in ways that were selectively toxic to tumor cells.

These studies initially demonstrated that lipoate analogs, designated thioctoids, had strong anti-cancer activity in cell culture [2; our unpublished results]. Many of our initial analyses were carried out using our current lead clinical investigational compound, CPI-613 (Figure 4). Indeed, these agents induce cell death in cancer cells extremely efficiently, with all cells dying within 24-48 hours by a mixture of apoptosis and necrosis-like mechanisms. This is in contrast to DCA which typically induces cell death in only a small fraction of tumor cells in culture [1; our unpublished results]. Each of the many human tumor cell lines we have examined is comparably sensitive to thioctoids, with tumor genotype at oncogenes and multidrug resistance loci having little or no effect on response [2].

Though the precise biochemical mechanism of action of thioctoids remains to be established, the *in vivo* behavior of these compounds demonstrates that they modulate PDK regulation of PDH [2; our unpublished results]. Moreover, these tumor effects are diametrically opposite to the effects of DCA and of other small molecule PDK inhibitors. Specifically, DCA induces *de-phosphorylation* of PDH E1 *in vivo* [55] while thioctoids induce *hyper-phosphorylation in vivo* [2; our unpublished results]. This thioctoid-induced PDH hyper-phosphorylation is selective for tumor cells and correlates with inactivation of PDH activity as assessed by analysis of the oxidative flux of pyruvate carbon through the complex. Coincident with this inhibition of PDH activity, thioctoids induce rapid shut down of mitochondrial ATP synthesis, typically reducing mitochondrial ATP production from pyruvate and glutamine carbon sources to less than 10% of controls within 15-30 minutes [2]. Finally, the capacity of CPI-613 to kill tumor cells is significantly attenuated in cell culture systems by RNAi knockdown of regulatory PDKs, supporting a role for PDKs in the response to these agents.

Collectively, these results indicate that thioctoid lipoate analogs kill tumor cells by addressing tumor-specific PDK regulation of PDH. Moreover, the consequence of these

effects is apparently to exploit tumor-specific PDH regulation, in part or entirely, to shut down tumor mitochondrial function. As continued mitochondrial function is required for the survival of tumor cells, even in the hypoxic solid tumor environment (above), these effects are expected to engender the observed response, rapid commitment to and execution of mitochondrially controlled cell death pathways.

Figure 4. Structure of thioctoid CPI-613 compared to biogenic lipoates. The structure of the thioctoid CPI-613 is shown at bottom. This molecule is a non-redox active analog of lipoate designed to have some resemblance to the acylated form of lipoate as it occurs in the normal catalytic cycle of PDH (Figure 2). The corresponding biogenic lipoate intermediates in the PDH catalytic cycle are illustrated at top.

These properties suggested that thioctoids might be promising anti-cancer agents. This possibility is further supported by their behavior in human tumor xenograft mouse models. Specifically, CPI-613 produces strong tumor growth inhibition in several tumor types. For example, the growth of BxPC-3 human pancreatic tumor xenografts implanted subcutaneously on posterior flanks is largely or entirely suppressed by intraperitoneal dosing with CPI-613 at 25-75mg/kg. Moreover, ca. 40% of these animals survive for more than 9 months (termination of study) without regrowth of tumors after an initial three week treatment regime. [A large fraction of the 60% mortality in this study is attributable to normal attrition in these genetically immunocompromised *nude* mice. Thus, cancer-free post-treatment survival in these animals is apparently substantially higher than 40%.]

Titration studies demonstrate that CPI-613 produces very substantial tumor growth inhibition in xenograft model systems at doses (0.1-1mg/kg) very substantially lower than the maximum tolerated dose (ca. 100mg/kg)[2; our unpublished results]. This indicates a very high possible therapeutic index. Large-animal toxicology studies further corroborate the very low toxicity of CPI-613 [69]. Moreover, drug metabolism studies indicate that CPI-613 breakdown products are unlikely to pose a barrier to clinical development [70].

Based on this favorable mechanistic and pre-clinical animal data, Phase I/II clinical trials are currently underway at several locations (see comments at <http://clinicaltrials.gov/ct2/show/NCT01520805>). These studies indicate that adverse events of CPI-613 in humans are low and mostly mild (well tolerated to at least 3,000mg/m², when infused over one hour) and suggest efficacy against several advanced cancers, including refractory/relapsed AML, in several patients [71-73]. It will be of interest to continue to pursue the clinical investigation of these investigational drugs.

7. The central role of lipoate as a metabolic regulatory signal, other potential thioctoid tumor targets

In view of the capacity of thioctoids to attack tumor cell PDH it may be of value to consider the potential of these agents to address tumor metabolism more generally. Indeed, the properties of lipoate suggest that it may act as a global mitochondrial regulatory signaling molecule, possibly addressing the entire flow of carbon through this compartment. On this view, lipoate analogs may address multiple regulatory pathways, some or all altered in tumor cells analogously to the well-understood PDH case.

Specifically, two other enzyme complexes are recognizably homologous to PDH and catalyze analogous reactions. One of these is the branched chain oxoacid dehydrogenase complex (BCDH) as reviewed in [74]. The other is the KGDH complex (above). BCDH governs the entry of carbon from a series of amino acids into the TCA cycle. KGDH governs the entry of glutamine-derived carbon (Figure 1). Together with PDH control of carbohydrate derived carbon, these three enzymes directly control initial access of all carbon to the TCA cycle, except for that derived from fatty acids. Moreover, even fatty acid-derived

carbon flow is implicitly governed by lipoate-dependent dehydrogenases because no fatty acid carbon can be oxidatively released without first passing through the KGDH complex [6]. Thus, all carbon flow through mitochondria is ultimately controlled by lipoate-containing enzymes.

The role of lipoate residues in controlling PDH function and the unique capacity of these residues to poll and reflect the mitochondrial energy status strongly suggests that lipoate residues will play a role in regulating BCDH and KGDH function. While this possibility remains to be investigated, it is noteworthy that the BCDH complex is regulated by kinases homologous to PDKs. It is plausible that these regulatory kinases are also responsive to lipoate redox/acetylation state. Moreover, in view of the rather dramatic reprogramming of tumor mitochondrial metabolism, it is also plausible that BCDH regulatory kinases may be reprogrammed in malignancy.

KGDH is apparently not regulated by PDK-like kinases and the details of its metabolic regulation remain to be investigated. However, the complex shows strong allosteric regulation by end products, consistent with important regulatory objectives [75,76]. Thus, it is likely that KGDH lipoates also participate in this regulation in some way that largely remains to be determined.

As reviewed in section 3 above, the lipoate residues of PDH, BCDH and KGDH exist as a cloud polling crucial features of the mitochondrial energy state, including the aggregate levels of free reducing potential (NADH) and of their respective acyl-CoA products. Moreover, these details of the mitochondrial energy state are likely to be among the state variables most informative about the moment-to-moment metabolic needs of the mitochondrion. Further, these regulatory goals of mitochondria are so substantially altered in tumor cells that we anticipate that all these potential lipoate-sensitive regulatory targets might be substantially altered in tumor cells, as they clearly are in the PDH case. Finally, the long boom-like structure of the enzyme linked lipoamide moieties (Figures 2) and their attachment to highly mobile E2 enzyme subdomains (Figure 3) raises an additional important regulatory possibility. It is conceivable that these actively polling lipoate residues are not merely reporting their results to their individual complexes but also to other mitochondrial consumers of regulatory information.

Collectively, these considerations suggest that thioctoid lipoate analogs may achieve their dramatic inhibition of tumor cell mitochondrial metabolism by addressing multiple, essential metabolic regulatory processes in a tumor-specific fashion. The promising pre-clinical and early clinical properties of thioctoid CPI-613 indicate that it will be of interest to explore this possibility in detail going forward.

8. Conclusions

The central role of lipoate-dependent dehydrogenases in governing carbon flow through mammalian mitochondria is reflected in their extensive regulation. Moreover, both empirical evidence and theoretical considerations indicate that the regulation of these

complexes is strongly controlled by the dynamic status of their lipoate residues, reflecting a moment-to-moment polling of the mitochondrial matrix. Finally, evidence and theory also indicate that some or most of these lipoate-dependent regulatory processes are very significantly altered in support of the substantial repurposing of tumor cell mitochondrial metabolism relative to its normal cell condition.

Collectively, these results indicate that the regulatory reprogramming of these dehydrogenases may represent a target-rich environment for developing new anti-tumor drugs that have the crucial properties that may be required to impart new efficacy to cancer chemotherapy – targets that are both essential to the malignant condition and non-redundant in their function. The preclinical and early clinical properties of agents directed at these targets support the possibility of useful promise in this therapeutic domain.

Author details

Paul M. Bingham* and Zuzana Zachar

Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY

Cornerstone Pharmaceuticals, 1 Duncan Drive, Cranbury, NJ

Acknowledgement

This work was largely supported by Cornerstone Pharmaceuticals, Inc. with additional support from the Carol M. Baldwin Breast Cancer Research Fund; Stony Brook University Center for Biotechnology, and from the Department of Biochemistry and Cell Biology and the School of Medicine at Stony Brook University. We are grateful to many colleagues for helpful discussions, including Robert Shorr, Robert Rodriguez, King Lee, Shawn D. Stuart, Sunita Gupta, and Alexandra Schauble. Both authors have a financial stake in Cornerstone Pharmaceuticals, Inc. the clinical developer of thiocetoids, including CPI-613.

9. References

- [1] Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Bonnet S, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED. (2007) A Mitochondria-K⁺ Channel Axis Is Suppressed in Cancer and its Normalization Promotes Apoptosis and Inhibits Cancer Growth. *Cancer cell* 11: 37-51.
- [2] Zachar, Z, Marecek, J, Maturo, C, Gupta, S, Stuart, S D, Howell, K, Bingham, P M (2011) Non-Redox-Active Lipoate Derivatives Disrupt Cancer Cell Mitochondrial Metabolism and Are Potent Anticancer Agents *In Vivo*. *J. mol. med.* 89: 1137-1148.
- [3] DeBerardinis, R J, Cheng, T (2010) Q's Next: the Diverse Functions of Glutamine in Metabolism, Cell Biology and Cancer. *Oncogene* 29: 313-324.

* Corresponding Authors

- [4] Nealson, K H, Conrad, P G (1999) Life: Past, Present and Future Philos trans r. soc Lond B biol sci, 354: 1923-1939.
- [5] Raymond, J, Segre, D (2006) The Effect of Oxygen on Biochemical Networks and the Evolution of Complex Life. Science, 311:, 1764-1767.
- [6] Garrett, R, Grisham, C M (2010) Biochemistry (4th ed) Belmont, CA: Brooks/Cole, Cengage Learning
- [7] Patel, M S, Korotchkina, L G (2006) Regulation of the Pyruvate Dehydrogenase Complex. Biochem. soc. trans., 34: 217-222.
- [8] Roche, T E, Hiromasa, Y (2007) Pyruvate Dehydrogenase Kinase Regulatory Mechanisms and inhibition in Treating Diabetes, Heart Ischemia, and Cancer. Cell. mol. life sci. 64: 830-849.
- [9] Hiromasa, Y, Hu, L Y, Roche, T E (2006) Ligand-Induced Effects on Pyruvate Dehydrogenase Kinase Isoform 2. J. biol. chem. 281: 12568-12579.
- [10] Perham, R N (2000) Swinging Arms and Swinging Domains in Multifunctional Enzymes: Catalytic Machines for Multistep Reactions Annu. rev.biochem., 69: 961-1004.
- [11] Garland, P B, Randle, P J (1964) Control of Pyruvate Dehydrogenase in Perfused Rat Heart by Intracellular Concentration of Acetyl-Coenzyme. A. biochem. j. 91: C6-C12.
- [12] Kanzaki, T, Hayakawa, T, Hamada, M, Fukuyoshi Y, Koike, M (1969) Mammalian Alpha-Keto Acid Dehydrogenase Complexes. IV. Substrate Specificities and Kinetic Properties of Pig Heart Pyruvate and 2-Oxoglutarate Dehydrogenase Complexes. J.biol. chem. 244: 1183-1187.
- [13] Linn, T C, Pettit, F H, Reed, L J (1969) Alpha-Keto Acid Dehydrogenase Complexes X Regulation of Activity of Pyruvate Dehydrogenase Complex From Beef Kidney Mitochondria by Phosphorylation and Dephosphorylation. Proc. nat. acad. sci. USA 62: 234-241.
- [14] Baker, J C, Yan, X H, Peng, T, Kasten, S, Roche, T E (2000) Marked Differences Between Two Isoforms of Human Pyruvate Dehydrogenase Kinase. J. biol. chem. 275: 15773-15781.
- [15] Roche, T E, Baker, J C, Yan, Y H, Hiromasa, Y, Gong, X M, Peng, T, Kasten, S A (2001) Distinct Regulatory Properties of Pyruvate Dehydrogenase Kinase and Phosphatase Isoforms. Prog. nucleic acid res. mol. biol., 70: 33-75.
- [16] Kato, M, Chuang, J L, Tso, S C, Wynn, R M, Chuang, D T (2005) Crystal Structure of Pyruvate Dehydrogenase Kinase 3 Bound To Lipoyl Domain 2 of Human Pyruvate Dehydrogenase Complex. Embo j. 24: 1763-1774.
- [17] Knoechel, T R, Tucker, A D, Robinson, C M, Phillips, C, Taylor, W, Bungay, P J, Brown, D G (2006) Regulatory Roles of the N-Terminal Domain Based on Crystal Structures of Human Pyruvate Dehydrogenase Kinase 2 Containing Physiological and Synthetic Ligands. Biochemistry, 45: 402-415.
- [18] Boulatnikov, I, Popov, K A (2003) Formation of Functional Heterodimers by Isozymes 1 and 2 of Pyruvate Dehydrogenase Kinase. Biochim. biophys. acta 1645: 183-192.

- [19] Pettit, F H, Pelley, J W, Reed, L J (1975) Regulation of Pyruvate-Dehydrogenase Kinase and Phosphatase by Acetyl-CoA-CoA and NADH-NAD Ratios. *Biochem. biophys. res. commun.* 65: 575-582.
- [20] Batenburg JJ, Olson MS. (1976) Regulation of Pyruvate Dehydrogenase by Fatty Acid in Isolated Rat Liver Mitochondria. *J. biol. chem.* 251: 1364-1370.
- [21] Hansford, R G (1976) Studies on Effects of Coenzyme A-Sh - Acetyl Coenzyme A, Nicotinamide Adenine-Dinucleotide - Reduced Nicotinamide Adenine-Dinucleotide, and Adenosine-Diphosphate - Adenosine-Triphosphate Ratios on Interconversion of Active and Inactive Pyruvate-Dehydrogenase in Isolated Rat-Heart Mitochondria. *J. biol. chem.* 251: 5483-5489.
- [22] Pratt, M L, Roche, T E (1979) Mechanism of Pyruvate Inhibition of Kidney Pyruvate-Dehydrogenase Kinase and Synergistic Inhibition by Pyruvate and ADP. *J. biol. chem.* 254: 7191-7196.
- [23] Bao, H Y, Kasten, S A, Yan, X H, Hiromasa, Y, Roche, T E (2004) Pyruvate Dehydrogenase Kinase Isoform 2 Activity Stimulated by Speeding Up the Rate of Dissociation of ADP. *Biochemistry* 43: 13442-13451.
- [24] Hiromasa, Y, Roche, T E (2003) Facilitated Interaction Between the Pyruvate Dehydrogenase Kinase Isoform 2 and the Dihydrolipoyl Acetyltransferase. *J. biol. chem.* 278: 33681-33693.
- [25] Cate, R L, Roche, T E (1978) Unifying Mechanism for Stimulation of Mammalian Pyruvate Dehydrogenase Kinase by Reduced Nicotinamide Adenine-Dinucleotide, Dihydrolipoamide, Acetyl Coenzyme-A, or Pyruvate. *J. biol. chem.* 253: 496-503.
- [26] Cate, R L, Roche, T E (1979) Function and Regulation of Mammalian Pyruvate-Dehydrogenase Complex - Acetylation, Interlipoyl Acetyl Transfer, and Migration of the Pyruvate-Dehydrogenase Component. *J. biol. chem.* 254: 1659-1665.
- [27] Korotchkina, L G, Patel, M S (2001) Site Specificity of Four Pyruvate Dehydrogenase Kinase Isoenzymes Toward the Three Phosphorylation Sites of Human Pyruvate Dehydrogenase. *J. biol. chem.* 276: 37223-37229.
- [28] Collins, J H, Reed, L J (1977) Acyl Group and Electron Pair Relay System - Network of Interacting Lipoyl Moieties in Pyruvate and Alpha-Ketoglutarate Dehydrogenase Complexes from *Escherichia coli*. *Proc. nat. acad. sci. USA* 74: 4223-4227.
- [29] Liu, S J, Baker, J C, Andrews, P C, Roche, T E (1995) Recombinant Expression and Evaluation of the Lipoyl Domains of the Dihydrolipoyl Acetyl Transferase Component of the Human Pyruvate -Dehydrogenase Complex. *Arch. biochem. biophys.* 316: 926-940.
- [30] Warburg, O (1930) *The Metabolism of Tumors* London, UK: Constable Press
- [31] Tennant, D A, Duran, R V, Gottlieb, E (2010) Targeting Metabolic Transformation for Cancer Therapy. *Nat. rev. canc.* 10: 267-277.
- [32] Cairns, R A, Harris, I S, Mak, T W (2011) Regulation of Cancer Cell Metabolism. *Nat. rev. canc.* 11: 85-95.

- [33] Vander Heiden, M G (2011) Targeting Cancer Metabolism: A Therapeutic Window Opens. *Nat. rev. drug disc.* 10: 671-684.
- [34] Ramsay, E E, Hogg, P J, Dilda, P J (2011) Mitochondrial Metabolism inhibitors for Cancer Therapy *Pharm. res.*, 28: 2731-2744.
- [35] Metallo, C M, Gameiro, P A, Bell, E L, Mattaini, K R, Yang, J J, Hiller, K, Stephanopoulos, G (2012) Reductive Glutamine Metabolism by IDH1 Mediates Lipogenesis Under Hypoxia. *Nature*, 481, 380-384.
- [36] Mullen, A R, Wheaton, W W, Jin, E S, Chen, P H, Sullivan, L B, Cheng, T, DeBerardinis, R J (2012) Reductive Carboxylation Supports Growth in Tumour Cells with Defective Mitochondria. *Nature*, 481: 385-388.
- [37] Elstrom, R L, Bauer, D E, Buzzai, M, Karnauskas, R, Harris, M H, Plas, D R, Thompson, C B (2004) Akt Stimulates Aerobic Glycolysis in Cancer Cells. *Canc. res.* 64: 3892-3899.
- [38] Wise, D R, DeBerardinis, R J, Mancuso, A, Sayed, N, Zhang, X Y, Pfeiffer, H K, Thompson, C B (2008) Myc Regulates a Transcriptional Program That Stimulates Mitochondrial Glutaminolysis and Leads To Glutamine Addiction. *Proc. nat. acad. sci. USA* 105: 18782-18787.
- [39] Wise, D R, Thompson, C B (2010) Glutamine Addiction: A New Therapeutic Target in Cancer. *Trends biochem. sci.* 35: 427-433.
- [40] DeBerardinis R J, Lum, J J, Thompson C B (2008) The Biology of Cancer: Metabolic Reprogramming Fuels Cell Growth and Proliferation. *Cell metabolism.* 7:11-20.
- [41] Tatum, J L, Kelloff, G J, Gillies, R J, Arbeit, J M, Brown, J M, Chao, K S C, Sullivan, D (2006) Hypoxia: Importance in Tumor Biology, Noninvasive Measurement by Imaging, and Value of its Measurement in the Management of Cancer Therapy. *int. j. rad. biol.* 82: 699-757.
- [42] Wilson, W R, Hay, M P (2011) Targeting Hypoxia in Cancer Therapy. *Nat. rev. canc.* 11(6), 393-410.
- [43] Dvorak HF (1986) Tumors: Wounds That Do Not Heal. Similarities Between Tumor Stroma Generation and Wound Healing. *N. engl. j. med.* 315: 1650-1659.
- [44] Schafer M, Werner S.(2008) Cancer as an Overhealing Wound: An Old Hypothesis Revisited. *Nature rev. mol. cell biol.* 9: 628-638.
- [45] Nagy JA, Chang SH, Dvorak AM, Dvorak HF. (2009) Why Are Tumour Blood Vessels Abnormal and Why Is It Important to Know? *Br. j. cancer.* 100: 865-869.
- [46] Insider, S (2010) UPDATED: A Skeptic Questions Cancer Genome Projects. *Science online*, <http://newssciencemagorg/scienceinsider/2010/04/a-skeptic-questions-cancer-genomhtml>
- [47] Stratton, M R (2011) Exploring the Genomes of Cancer Cells: Progress and Promise. *Science*, 331: 1553-1558.
- [48] MacDougall, R (2012) Bert Vogelstein Considers the Cancer Genome at 10th Annual NHGRI Trent Lectureship National Human Genome Research institute, <http://www.genome.gov/27547785>

- [49] Vaidya, S., K. Ghosh, et al. (2011). Recent Developments in Drug Resistance Mechanism in Chronic Myeloid Leukemia: A Review. *European Journal of Haematology* 87(5): 381-393
- [50] Koukourakis, M I, Giatromanolaki, A, Sivridis, E, Gatter, K C, Harris, A L, Tumor Angiogenesis Res, G (2005) Pyruvate Dehydrogenase and Pyruvate Dehydrogenase Kinase Expression in Non-Small Cell Lung Cancer and Tumor-Associated Stroma. *Neoplasia*, 7: 1-6.
- [51] Lu, C W, Lin, S C, Chien, C W, Lee, C T, Lin, B W, Lee, J C, Tsai, S J (2011) Overexpression of Pyruvate Dehydrogenase Kinase 3 Increases Drug Resistance and Early Recurrence in Colon Cancer *American j. pathol.*, 179: 1405-1414.
- [52] Kim JW, Tchernyshyov I, Semenza GL, Dang CV.(2006) HIF-1-Mediated Expression of Pyruvate Dehydrogenase Kinase: A Metabolic Switch Required for Cellular Adaptation to Hypoxia. *Cell metabolism*. 3: 177-185.
- [53] Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. (2006) HIF-1 Mediates Adaptation to Hypoxia by Actively Downregulating Mitochondrial Oxygen Consumption. *Cell metabolism* 3: 187-197.
- [54] Hanahan, D, Weinberg, R A (2011) Hallmarks of Cancer: The Next Generation. *Cell* 144: 646-674.
- [55] Harris, A L (2002) Hypoxia - A Key Regulatory Factor in Tumour Growth. *Nat. rev. canc.* 2: 38-47.
- [56] McFate, T, Mohyeldin, A, Lu, H, Thakar, J, Henriques, J, Halim, N D, Verma, A (2008) Pyruvate Dehydrogenase Complex Activity Controls Metabolic and Malignant Phenotype in Cancer Cells. *J.biol. chem.* 283: 22700-22708.
- [57] Lu, H S, Forbes, R A, Verma, A (2002) Hypoxia-Inducible Factor 1 Activation by Aerobic Glycolysis Implicates the Warburg Effect in Carcinogenesis. *J.biol. chem.* 277: 23111-23115.
- [58] Lu, C W, Lin, S C, Chen, K F, Lai, Y Y, Tsai, S J (2008) Induction of Pyruvate Dehydrogenase Kinase-3 by Hypoxia-Inducible Factor-1 Promotes Metabolic Switch and Drug Resistance. *J.biol. chem.* 283: 28106-28114.
- [59] Visch, H J, Koopman, W J H, Zeegers, D, Vries, S, van Kuppeveld, F J M, van den Heuvel, L, Willems, P (2006) Ca²⁺-Mobilizing Agonists increase Mitochondrial ATP Production to Accelerate Cytosolic Ca²⁺ Removal: Aberrations in Human Complex I Deficiency. *Am. j. physiol. cell physiol.*, 291: C308-C316.
- [60] Krampe, B, Al-Rubeai, M (2010) Cell Death in Mammalian Cell Culture: Molecular Mechanisms and Cell Line Engineering Strategies. *Cytotechnology*, 62: 175-188.
- [61] Nagley, P, Higgins, G C, Atkin, J D, Beart, P M (2010) Multifaceted Deaths Orchestrated by Mitochondria in Neurons. *Biochim. biophys. acta*, 1802: 167-185.
- [62] Stacpoole, P W, Kerr, D S, Barnes, C, Bunch, S T, Carney, P R, Fennell, E M, Valenstein, E (2006) Controlled Clinical Trial of Dichloroacetate for Treatment of Congenital Lactic Acidosis in Children. *Pediatrics*, 117: 1519-1531.

- [63] Bowker-Kinley, M M, Davis, W I, Wu, P F, Harris, R A, Popov, K M (1998) Evidence for Existence of Tissue-Specific Regulation of the Mammalian Pyruvate Dehydrogenase Complex. *Biochem. j.* 329: 191-196.
- [64] Cao, W G, Yacoub, S, Shiverick, K T, Namiki, K, Sakai, Y, Porvasnik, S, Rosser, C J (2008) Dichloroacetate (DCA) Sensitizes Both Wild-Type and over Expressing Bcl-2 Prostate Cancer Cells *in Vitro* To Radiation. *Prostate* 68: 1223-1231.
- [65] Wong, J Y Y, Huggins, G S, Debidda, M, Munshi, N C, De Vivo, I (2008) Dichloroacetate induces Apoptosis in Endometrial Cancer Cells. *Gynecol. oncol.* 109: 394-402.
- [66] Michelakis, E D, Sutendra, G, Dromparis, P, Webster, L, Haromy, A, Niven, E, Petruk, K C (2010) Metabolic Modulation of Glioblastoma With Dichloroacetate. *Sci. transl. med.*, 2: 31-34.
- [67] Khan, A (2011) Use of Oral Dichloroacetate for Palliation of Leg Pain Arising From Metastatic Poorly Differentiated Carcinoma: A Case Report. *J. palliat. med.*, 14: 973-977.
- [68] Brandsma, D, Dorlo, T P C, Haanen, J H, Beijnen, J H, Boogerd, W (2010) Severe Encephalopathy and Polyneuropathy Induced by Dichloroacetate. *J. neurol.* 257: 2099-2100.
- [69] Lee K, Maturo C, Shorr R, Rodriguez R. (2010) Uncommon Toxicologic Profile at Toxic Doses of CPI-613 (an Agent Selectively Alters Tumor Energy Metabolism) in Rats and Minipigs Reflects Novel Mechanism. *American Journal of Pharmacology and Toxicology* 5: 183-208.
- [70] Lee, K, Shorr, R, Rodriguez, R, Maturo, C, Boteju, LW and Sheldon A (2011) Formation and Anti-Tumor Activity of Uncommon *in Vitro* and *in Vivo* Metabolites of CPI-613, A Novel Anti-Tumor Compound that Selectively Alters Tumor Energy Metabolism. *Drug metab. lett.* 5: 163-182.
- [71] Pardee TS, Levitan DA, Hurd DD: (2011) Altered Mitochondrial Metabolism as a Target in Acute Myeloid Leukemia [abstract]. *J Clin Oncol* 29: suppl; abstr 6590
- [72] Pardee TS, DeFord-Watts LM, Peronto E, Levitan D, Hurd D, Kridel SK, Powell B. (2012) The 1st in Class Tumor Specific Anti-Mitochondrial Metabolism Agent CPI-613 is Well Tolerated and Has Activity in Several Hematologic Malignancies [abstract]. *J clin oncol* 30: suppl
- [73] Lee K, Khaira D, Rodriguez R, Maturo C, O'Donnell K, Shorr R. (2011) Long-Term Stable Disease of Stage IV Pancreatic Neuroendocrine Tumors and Without Significant Adverse Effect by CPI-613, an Investigational Novel Anti-Cancer Agent. *Case Study and Case Report*, 1:137-145.1
- [74] Harris RA, Joshi M, Jeoung NH, Obayashi M. (2005) Overview of the Molecular and Biochemical Basis of Branched-Chain Amino Acid Catabolism. *J. nutr.* 135:1527S-1530S
- [75] Strumilo, S (2005) Short-Term Regulation of the Alpha-Ketoglutarate Dehydrogenase Complex by Energy-Linked and Some Other Effectors. *Biochemistry-Moscow*, 70: 726-729.

- [76] Bunik, V I, Fernie, A R (2009) Metabolic Control Exerted by the 2-Oxoglutarate Dehydrogenase Reaction: A Cross-Kingdom Comparison of the Crossroad Between Energy Production and Nitrogen Assimilation. *Biochem. j.* 422: 405-421.

IntechOpen

IntechOpen