

# Lipoic acid and lipoic acid analogs in cancer metabolism and chemotherapy

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**ABSTRACT:** The lipoic acid (lipoate) coenzyme is unique in all of mammalian metabolism. It is not only crucial to the function of some of the major enzymes feeding carbon into the tricarboxylic acid (TCA) cycle, it also generates dynamic regulatory information about the metabolic status of the mitochondrial matrix ultimately functioning to control these metabolic fluxes. Moreover, these lipoate-sensitive regulatory processes are apparently systematically redesigned in tumor cells and the affected enzymes commonly become especially central to cancer metabolism. Thus, lipoate-sensitive regulatory processes constitute potentially uniquely valuable targets for chemotherapeutic attack. Our goal here is to review

the current status of our knowledge relevant to the use of lipoate and lipoate analogs to therapeutically attack malignant disease.

**KEY WORDS:** lipoic acid, lipoate, lipoic acid analogs, lipoate analogs, cancer metabolism, tumor mitochondria, two compartment metabolism, chemotherapy

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## Introduction

Lipoic acid (1,2-dithiolan-3-pentanoic acid; lipoate or LA) is an 8-carbon organosulfur compound containing sulfur substitutions to the 6- and 8-carbons (Figures 1 & 2). Lipoate was first isolated by Reed and colleagues in 1951 in one of the many heroic, indeed, Herculean, efforts so characteristic of the classical era of biochemistry. This discovery and initial characterization involved the processing of approximately 10 tons of bovine liver to yield 30 mg of lipoate [1].

In the ensuing 63 years, the relatively small community of lipoate investigators has substantially advanced our understanding of the biological role of this molecule [see 2 for an historical review]. Lipoate acts as a covalently joined cofactor for several enzymes, including those that typically govern most of the flow of carbon into mitochondrial metabolism in tumor cells. Moreover, in the course of the reversible covalent modification of lipoate in its catalytic role (Figure 1A), this molecule also reflects crucial regulatory information (Figure 2C). As this information is interpreted differently in tumor and normal cell mitochondria (below), lipoate-responsive regulatory processes are uniquely centrally and strategically placed for selective chemotherapeutic attack on cancer.

We will review features of the biochemistry of lipoate and of attempts to use the biogenic lipoate molecule as an anti-cancer agent. While lipoate has shown indications of clinical efficacy in treating potentially redox-sensitive disorders, such as diabetic complications and Alzheimer's disease [3,4], there is relatively little preclinical indication of potential for this molecule in cancer therapy. In contrast, we will review our work indicating that properly designed xenobiotic, non-redox-active analogs of lipoate can act as potent chemotherapeutic agents, selectively attacking tumor cell mitochondrial metabolic control, resulting in cancer cell death. These results indicate that lipoate analogs may hold substantial clinical promise as agents to attack the uniquely situated targets of lipoate-sensitive tumor cell metabolic regulation.

## **Lipoate, fundamental biochemistry and use as a dietary supplement**

Lipoate in context: There are five currently known lipoate-using enzyme complexes in mammals. These are pyruvate dehydrogenase (PDH), alpha-ketoglutarate dehydrogenase (KGDH), the branched chain alpha-keto acid dehydrogenase (BCKDH), oxoadipate dehydrogenase (OADH), and the glycine cleavage system (GCS) [reviewed in 5]. The 6-carbon of lipoate is a chiral center, thus lipoate exists as both R- and S- enantiomer, though only the former is found in nature.

Lipoate sulfhydryls undergo cyclic, reductive and oxidative covalent modification in the course of enzymatic catalysis (Figure 1). Lipoate is attached covalently to a specific subunit(s) in each of the five complexes through a non-peptide amide linkage between the lipoate carboxyl group and the epsilon amino group of a specific active-site lysine. The combined length of the lysine side chain and the lipoate backbone results in a molecular boom that swings between the active sites of the enzyme complex components to allow the sequence of reactions (Figure 1; below)

Lipoate as a biological redox agent: Though naturally occurring levels of free lipoate are extremely low in the body and in unprocessed foods, purified lipoate can be consumed as a dietary supplement, substantially elevating free lipoate levels (below). One of the most likely effects of elevated lipoate levels is modulation of redox metabolism.

Lipoate was first proposed to act as an antioxidant in 1959, when administration of LA (the oxidized form of lipoate; Figure 2A) was found to ameliorate vitamin C and E deficiencies in laboratory guinea pigs and rats, respectively [6]. It was not until the last three decades and the realization of the

importance of redox control and signaling that the specific antioxidant mechanisms and targets of LA and its reduced counterpart dihydro-lipoic acid (DHLA; Figure 2A) were elucidated. LA and DHLA have been found to chelate heavy metals [7], scavenge various reactive oxygen species [3], increase and/or recharge other cellular antioxidant pools [8], and affect antioxidant gene expression [9].

Owing at least in part to LA's constrained dithiolane ring (Figure 2A), the redox potential of the LA/DHLA couple make it among the most potent antioxidants found in nature at -0.32V [10]. It is a more effective antioxidant than similar sulfur-containing redox pairs such as GSSG/GSH (-0.24V) and cystine/cysteine (-0.22V) [11]. Moreover, DHLA has been found to directly reduce cystine, indirectly leading to increases in cellular GSH, as well as to regenerate antioxidants such as vitamins C and E, further enhancing cellular antioxidant defenses [8].

While cystine reduction appears to predominantly take place extracellularly, both R- and S-LA have been shown to be taken up by cells either in a pH-dependent manner, via the actions of the monocarboxylate transporters, or via the Na<sup>+</sup>-dependent vitamin transporters [12, 13]. Once in the cytosol, LA is reduced to DHLA by NAD(P)H-dependent enzymes such as thioredoxin reductase, glutathione reductase, and dihydrolipoamide dehydrogenase (the E3 subunit of the 2-oxoacid dehydrogenases; Figure 1A). Following enzymatic reduction, up to 98% of total DHLA produced is re-exported from the cell, though intracellular concentrations remain stable at approximately 1mM in the presence of substantial extra cellular concentrations [14].

Only the R-LA stereoisomer is found ligated to lipoate-containing enzymes (Figures 1 & 2; below). However, both enantiomers (and their reduced counterparts) are effective scavengers of various reactive oxygen species (ROS) when administered exogenously. While DHLA has also been shown to scavenge hydroxyl and hypochlorous radicals, it likely does not react with singlet oxygen. However, unlike LA, DHLA has shown the ability to scavenge superoxide radicals [3]. Notably, neither LA nor DHLA are

able to directly scavenge hydrogen peroxide, arguably the most abundant second messenger ROS, in the absence of enzymatic catalysis.

Lipoate as a dietary supplement: Lipoate's high antioxidant activity, combined with its bioavailability and relatively low toxicity, has made it a popular candidate as a nutritional supplement and as a candidate pharmacotherapeutic agent. LA has been investigated as treatment for neurodegenerative and age-related diseases such as Alzheimer's Disease (AD), where it has shown signs of decreasing plaque formation and lipid peroxidation, resulting in increased cellular antioxidant levels and increased mitochondrial function in animal models [15]. Human clinical data are limited, but suggest LA treatment may reduce mental decline in patients with moderate AD [4].

The most abundant human data on human dietary LA supplementation is from clinical studies on diabetic polyneuropathy in Germany, where it is an approved treatment option. A number of studies have shown that LA administered orally, intravenously, or intramuscularly has resulted in significant improvement in a number of disease markers including lowered levels of oxidative stress-related products, increased levels of antioxidants, as well as amelioration of neuropathic pain [3]. It has been suggested that these improvements may be a result of LA-associated increased antioxidant capacity. Alternatively, LA may improve glucose uptake as a result of increased glucose transport due to its activation of AMPK kinase and effects on the Akt signaling in the insulin receptor pathway [9] through incompletely understood (but potentially redox) mechanisms.

Lipoate regulatory activity in enzymatic context: In addition to its role as an antioxidant, there is extensive evidence that enzyme-bound LA and DHLA play a significant role in direct regulation of metabolic enzymes (also see below). Moreover, exogenous free R-LA has been shown to modulate the activities of the regulatory PDH Kinases (PDK1-4), resulting in PDH activation [16]. In the case of KGDH,

Bunik and colleagues [17] have shown that a thiyl-radical of E2-bound LA (probably generated by superoxide radical from the E3 subunit of KGDH in times of low NAD<sup>+</sup>/NADH ratios; below) is responsible for the irreversible oxidative inactivation of the E1 subunit. Interestingly, this E2 thiyl radical can be prevented from inactivating the E1 component through the actions of thioredoxin, which stabilizes the thiyl-LA intermediate, but with the added effect of stimulating further ROS generation. This thioredoxin-dependent protection of E1 may allow for the continued production of succinyl-CoA to fuel substrate-level phosphorylation during periods of low NAD<sup>+</sup> (e.g., during periods of mitochondrial oxidative phosphorylation dysfunction)[18].

The Szweda group has recently shown the E2-bound LA of KGDH to also be susceptible to inhibition by reversible glutathionylation in response to oxidative stress [19]. While the mechanistic significance of glutathionylation is not yet decisively established, these authors propose that this derivatization protects the sulfurs of LA from 'overoxidation' during periods of oxidative stress. LA sulfurs oxidized to sulfenic acids (-SOH) can be restored to the functional oxidation state via the actions of glutaredoxins or thioredoxins. However, when allowed to persist in times of high ROS concentrations, further oxidation to sulfinic (SOOH) or sulfonic (SO<sub>2</sub>OH) acids can take place, (apparently) irreversibly inhibiting enzymatic function.

Finally, since the 2-oxoacid dehydrogenases (in particular KGDH), are known to also be *generators* of ROS [20, 21], and the likely placement of KGDH in a supramolecular metabolon with other TCA enzymes [22], a picture is emerging of KGDH as a major sensor of mitochondrial metabolic health [23], including through redox self-modulation (below).

Summary: Lipoate, by way of its direct and indirect effects on cellular antioxidant systems and in situ regulation of cellular metabolism is a molecule of significant interest across a wide range of areas of investigation. The very important question remains as to which of the observed clinical and preclinical

effects of exogenous lipoate are due to this molecule's potent redox activity and which, if any, may result from regulatory effects of interaction with machinery normally designed to respond to the various covalent intermediates in the lipoate catalytic cycle. We turn further attention to these questions below.

## **Lipoate, preclinical studies of the biogenic form of lipoate as a cancer chemotherapeutic agent**

Lipoate as a clinical agent: In 1966 German physicians reported reduced levels of lipoate in patients with liver cirrhosis, diabetes mellitus, and polyneuropathy and treated these patients with lipoate acid supplements [reviewed in 24]. This ultimately led to the investigation of lipoate as a therapeutic agent in various conditions where oxidative stress was implicated as part of the inherent pathology. Initially, this focused on diabetes mellitus and liver cirrhosis. The recognition of the altered redox state of tumors and tumor cells [reviewed in 24 and 25] subsequently resulted in the investigation of the therapeutic potential of lipoate as a redox management agent in various human tumor cell and animal models. We briefly review here the relevant findings of these preclinical studies.

Cultured tumor cell models: Studies have been performed in established cultured human tumor cells derived from breast, colon, hepatic, lung, lymphoid, neural, ovarian and thyroid cancers (studies below). Additionally, a few studies were also performed in artificially transformed cell models, including K-ras transformed 3T3 cells [26] and H-ras transformed 3Y1 rat fibroblasts [27]. In the majority of these studies, the effects of lipoate on inhibiting proliferation and/or inducing apoptosis were investigated. Lipoate doses tested ranged very widely, from 0.1mM to 10mM. In all inhibition of proliferation studies only doses of 1mM or greater inhibited proliferation and these by 50% or less and only after 48-72 hours of treatment [28, 29, 30]. In studies where lipoate was tested for its ability to induce apoptosis [23, 28,

31, 32] treatments with 1mM or higher for up to 72 hours were reported. Levels of apoptosis range from 90% of cells after 72 hours of 5mM treatment in FaDu head and neck cancer-derived tumor cells [26, 33] to 30% apoptotic cells after 24-48 hours of treatment with 1-2mM lipoate [33]. Only one study [34] reported induction of apoptosis in about 25% of cells after 24hr treatment with 0.1mM lipoate.

In a subset of the above studies, the effect of LA or DHLA on the production of reactive oxygen species (ROS) was examined [27, 31, 34]. Treatment with lipoate increased the production of  $O_2^{\cdot-}$  (superoxide) and/or  $H_2O_2$  (hydrogen peroxide) [31, 32]. Pretreatment of cells with antioxidants such as N-acetyl-cysteine (NAC) ameliorated ROS production. These reports of ROS *production* in response to lipoate treatment led to the insight that lipoate can sometimes act as a pro-oxidant, in addition to its previously recognized anti-oxidant effects. We will return to this last observation again below in the context of xenobiotic lipoate analogs.

Finally, several studies examined the effect of lipoate treatment on gene expression in some tumor cell models. Various investigators explored components of the apoptotic machinery and found down regulation of Bcl in HT29 cells [31], H460 cells [34] and MCF7 cells [33] in response to lipoate treatment. Several investigators found that lipoate treatment reduced proliferation in FaDu, Hey8A and MCF7 cells [26, 33, 35] and correlated with stabilization of the cyclin-dependent kinase inhibitor p27kip1 in FaDu cells [26], Hey8A cells [35] and MCF7 cells [33].

Lee et al. [29] investigated lipoate effects on the invasive capabilities of MDA-MB 231 breast tumor cells in an in vitro model system and found down regulation of two key metalloproteinases-2 and -9. Choi et al. [30] investigated the effects of lipoate on de-differentiated, iodine resistant thyroid cancer (TPC-1) cells and found up regulation of the CREB transcription factor and the iodine symporter, NIS. Elangovan & Hsieh (2008) describe the translocation of the transcription factor Nfr2 to the nucleus and concomitant up-regulation of quinone reductase NQO1 in HL-60 cells.

Finally, Guerriero et al. [36] investigated the effects of lipoate treatment in two hepatoma cell lines, HepG2 and Huh7, on the expression of cytokines. They found down-regulation of pro-inflammatory cytokines TNF- $\alpha$ , IL-8 and IL-1 $\beta$  and up-regulation of the anti-inflammatory cytokine IL-10.

The concentrations of lipoate at which effects on gene expression were seen were quite high, ranging from 0.5 to 5mM with treatment times up to 72 hours. The magnitude of the effects correlated with dose and length of treatment. These high concentrations suggest that direct redox modulation by redox-active lipoate is likely to be an important component of the mechanisms in these cases.

Mouse models: There are a limited number of studies in which lipoate was used in human tumor xenograft models. Feurecker et al. [37] used human SkBr3 breast tumor xenografts. The treatment regime was a daily intraperitoneal injection at 18.5mg/kg for 22 days, starting on day 7 post tumor cell inoculation. They report about 75% reduction in tumor volume in treated mice compared to mock treated controls. Additionally, PET imaging of tumors in treated mice suggested a decrease in glucose uptake compared to mock treated controls.

Al Abdan [38], studied Ehrlich carcinoma cells inoculated intraperitoneally. Lipoate was administered orally at 50mg/kg, starting 1 day post inoculation and continued daily for 30 days. At 14 days post start of treatment, 4 mice of 20 were alive in the mock treated group compared to 14 mice of 20 alive in the LA treated group. At 30 days of treatment there were no survivors in the mock treated group and 2 survivors in the treated group. Additionally, the ascites volume was reduced about 3 fold in the LA treated group compared to the mock treated control.

Schwartz et al. [39] investigated the effectiveness of lipoate treatment in combination with hydroxycitrate in a syngeneic mouse bladder cancer model. One control group was treated with lipoate alone at 10mg/kg twice a day intraperitoneally. This group exhibited modest tumor growth inhibition after 24 days of treatment compared to the solvent controls.

Clinical trials: Berkson et al. [40] have reported four case studies where lipoate treatment in combination with other antioxidants and naltrexone resulted in apparent stabilization of disease in pancreatic cancer patients.

Summary comments on studies with the biogenic lipoate molecule: The majority of cultured cell studies saw substantial effects of lipoate treatment only at doses of 1mM or higher. These doses are high enough to significantly affect the cellular redox environment. Many investigators have explored and documented the effects of lipoate on cellular redox status and its consequences on cell signaling and gene transcription [reviewed in 41]. Thus, a plausible hypothesis accounting for the above reviewed lipoate effects on cell proliferation and changes in protein expression is that the effects of lipoate treatment are the consequence of changes in tumor cell redox status. This interpretation is supported by studies where the use of antioxidants such as N-acetyl cysteine, vitamin C, catalase, or MnTBAP significantly ameliorated effects of lipoate on apoptosis induction, changes in protein expression and ROS generation [32, 34]. The potential effects in the one set of clinical case studies may also have such a mechanistic basis.

In contrast to redox-active, biogenic lipoate, we review below evidence from our group that non-redox active lipoate analogs of appropriate design can have large effects on tumor cells in vitro and in vivo at doses much lower than required with the parental compound. These results indicate that lipoate analogs can address lipoate-sensitive regulatory processes that the parental lipoate molecule cannot address efficiently.

## **Non-redox active lipoate analogs attack lipoate-sensitive metabolic regulatory processes selectively in tumor cells**

Additional insight into the unique position of lipoate in tumor metabolism: It has recently become clear that the fundamentally altered metabolism of solid tumors is probably more complex than initially appreciated on the basis of the classic observations of Warburg, et al. [42]. This foundational early work revealed that solid *tumors* (not necessarily tumor cell lines) metabolized significant fractions of glucose to lactate, even in the presence of sufficient molecular oxygen to oxidize this glucose to carbon dioxide and water (aerobic glycolysis). The initial interpretations of Warburg's studies widely assumed that aerobic glycolysis was a property of the carcinoma cell portion of solid tumors, leading to extensive exploration of the Warburg effect in cultured carcinoma cells [see, for example, 25, 43, 44 and 45 for reviews of this perspective]. However, it is becoming increasingly clear that this perspective is sometimes, perhaps even usually, seriously incomplete.

Specifically, as we discuss in more detail below, a large body of recent evidence indicates that solid tumors sometimes display "two compartment metabolism." Tumor stromal cells (especially tumor fibroblasts) process glucose glycolytically, secreting lactate to be taken up by epithelial carcinoma cells for oxidative mitochondrial metabolism. Under these conditions, carcinoma cells are highly dependent on oxidative mitochondrial metabolism, both for energy generation (catabolism) and for production of several building block molecules needed for macromolecular synthesis (anabolism). Moreover, the poorly vascularized solid tumor environment requires that this vital mitochondrial metabolism be regulated differently than in most normal cells, perhaps coopting (in an altered, unregulated fashion) the corresponding machinery designed for normal wound healing [46, 47].

The metabolic enzyme-bound lipoate coenzyme is central to this re-regulated tumor mitochondrial metabolism, providing a new and potentially potent window to attack tumors. Consistent

with this prospect, we and our collaborators have shown that properly designed lipoate analogs, apparently mimicking normal catalytic intermediates, exert powerful effects through the reconfigured, lipoate-sensitive regulatory machinery of lipoate-using mitochondrial enzymes, driving tumor cell death.

Relevant details of two compartment metabolism in tumors: The seminal work of Lisanti and colleagues [reviewed in 48, 49, 50, 51] has revealed that two compartment metabolism is a common property of many solid tumors, correlating especially with advanced tumors having poor prognosis. This insight has stimulated broad interest in reinvestigating the Warburg effect and tumor metabolism with improved perspective.

Several lines of evidence supporting this picture of advanced tumor metabolism have emerged.

First, immunohistochemical analysis of solid tumor biopsy samples allows the efficient exploration of the metabolic configuration of tumor cell subpopulations. Of particular importance are the tumor-associated fibroblasts, making up a substantial portion of the tumor mass in most carcinomas, and their relationship to neighboring carcinoma cells. A variety of approaches have been taken to addressing this question. For example, there are several monocarboxylate transporters (MCTs) in mammals. MCT-1 is more efficient at importing lactate, while MCT-4 is more efficient at lactate export [50]. In a variety of advanced solid tumor biopsy samples, MCT-4 is substantially upregulated in stromal cells (indicating specialization for lactate export) while MCT-1 is upregulated in tumor cells (lactate import specialization).

An analogous approach is to examine the levels of mitochondrial function, using antibody probes against elements of the electron transport chain (ETC) like cytochrome oxidase [50 and references therein]. These probes produce the predicted two-compartment pattern, with elevated mitochondrial biogenesis in tumor cells and depressed mitochondrial levels in stromal cells in solid tumors.

Second, as mentioned, the extent of development of this two-compartment metabolic phenotype is strongly correlated with clinical outcome. An initial observation leading to this work was the stromal depression in the levels of caveolin, a protein associated with vesicle formation at the plasma membrane. This stromal caveolin reduction is currently thought to result from elevation in autophagy in these cells, part of their adaptation to acting as “feeders” for the epithelial carcinoma cells. As a result, stromal caveolin levels appear to be a reliable proxy for stromal commitment to two compartment metabolism. Stromal caveolin levels have proven to be very strong, independent predictors of clinical outcome in multiple tumor types, including breast and prostate based on studies from several groups [reviewed in 48].

Third, Lisanti and colleagues make a strong case that they can recapitulate some features of two compartment metabolism in cell culture by co-culture pairing of a tumor cell line with a fibroblast line. Under these conditions the tumor cells induce a reduction in mitochondrial metabolism and an elevation in glycolysis (assayed as above) in stromal cells. Reciprocally, these “activated” fibroblasts (apparently resembling tumor fibroblasts) induce increased mitochondrial biosynthesis and oxidative metabolism in the paired tumor cells [reviewed in 48, 49, 50, 51]. At present, the signal from tumor cells to fibroblasts appears to include ROS (especially hydrogen peroxide) and from activated stroma to tumor cells to include lactate, itself, creating a positive feedback circuit [reviewed in 48].

Fourth, this two compartment pattern is replicated in some normal, non-malignant contexts. For example, astrocytes and glial cells in mature brain tissue appear to metabolize glucose providing lactate to neurons for oxidative metabolism [reviewed in 48]. It is plausible to speculate that similar role differentiation might occur between mesenchymal and epithelial cells in wound healing, perhaps to be coopted in malignant transformation.

In summary, a substantial body of evidence indicates that solid tumor metabolism involves preferential glycolytic flux in stromal fibroblasts and preferential oxidative mitochondrial metabolism in

carcinoma cells themselves. Moreover, this metabolic pattern appears to become more extremely developed with tumor progression. Learning to target tumor metabolism with greater efficacy in vivo will likely require that we strategically attack two compartment metabolism. Targeting tumor cell mitochondrial metabolism is an attractive option for such an attack.

Lipoate-using enzymes and their regulation in tumor cells: The initial reactions catalyzed in each of the five lipoate-containing complexes are oxidative decarboxylation of the corresponding alpha-carboxyl substrate (Figure 1A). The resulting activated, partially oxidized residue of the substrate is then transferred directly to a lipoate sulfur. The accepting lipoate begins the catalytic cycle in the fully oxidized state represented by a disulfide bond between its two sulfurs, creating a five member aliphatic ring (Figure 1A). Lipoate reception of the activated substrate residue from the first reaction is associated with its reduction, opening the ring to produce a reduced sulfhydryl on one sulfur and a thioester or thioether on the other sulfur.

This lipoate-bound chemical unit is then transferred by the second activity in the complex to an appropriate acceptor (either Coenzyme-A or tetrahydrofolate; below), leaving a fully reduced lipoate containing two sulfhydryls (Figures 1 and 2A). This catalytic cycle is completed by the transfer of this lipoate reducing potential to  $\text{NAD}^+$ , generating NADH, by the final component of each complex (Figure 1A). [It is currently thought that the same enzyme (dihydrolipoate dehydrogenase, DLD) carries out this terminal electron transfer reaction in all five complexes, though there remains some ambiguity about whether this role might be played by an alternative oxidoreductase in some contexts in the glycine cleavage system [5].]

The two lipoate-using enzyme complexes that will concern us here are pyruvate dehydrogenase (PDH) and alpha-ketoglutarate dehydrogenase (KGDH), which import glucose/lactate-derived pyruvate (PDH) or glutamine-derived alpha-ketoglutarate (KGDH), respectively, into the TCA cycle. Glucose

glycolytic product (pyruvate/lactate) and glutamine-derived alpha-ketoglutarate account for the vast majority of carbon entering tumor mitochondrial metabolism. Thus, PDH and KGDH must be regulated properly for each context in which these mitochondria find themselves.

PDH is more thoroughly studied than KGDH, including its tumor mitochondrial re-regulation [reviewed in 44]. Specifically, a major route for regulation of PDH is through a set of four specific dimeric kinases (pyruvate dehydrogenase kinases, PDK1 through PDK4). These kinases have different properties, regulating the first enzyme of the complex (E1, pyruvate decarboxylase) through inactivating phosphorylations in response to various allosteric modifiers. The catalytic intermediates of lipoate are particularly important allosteric modifiers of at least some of these PDKs.

The four PDK isoforms are expressed in a tissue specific manner [52]. PDK1 and 3 are often upregulated in tumors, in contrast to the dominance of PDKs 2 and 4 in the bulk of normal adult tissues [53]. The relative amounts of the four PDK isoforms changes by as much as an order of magnitude during malignant transformation, in processes regulated by hypoxia inducible factor (HIF) and other pathways [54, 55]. Thus, we anticipate that the regulatory role and logic of lipoate catalytic intermediates in PDH will differ substantially in carcinoma cells compared to most normal cells.

KGDH regulation is less well characterized than PDH [see 56 and references therein]. There appear to be no kinases associated with the KGDH complex, so that its regulation will differ fundamentally from PDH. Several lines of evidence indicate that KGDH is likely to be regulated by a redox-dependent autoregulatory feedback process in which hydrogen peroxide produced as a minority product of the final redox step in the reaction. Transfer of reducing potential normally targeted to NAD<sup>+</sup> (Figure 1A) instead partially reduces molecular oxygen to superoxide which is rapidly dismutated to hydrogen peroxide. Superoxide and/or hydrogen peroxide then reversibly inactivates the E2 component (and possibly also E1) by redox modification, including modification of the sulfhydryls on the catalytic E2

lipoate. Moreover, our recent work indicates that this redox autoregulation responds to lipoate status and is substantially altered in tumor cells [56, below].

This strong regulatory role of lipoate intermediates is not surprising. Both PDH and KGDH are large complexes, having many copies of their component enzymes. This organization results in many copies of lipoate (more than 100 in the case of PDH) in the same complex. Moreover, the terminal reactions of PDH and KGDH are fully reversible (Figure 1A), making the acylation and redox status of the lipoates in PDH and KGDH highly sensitive to the overall metabolic status of the entire mitochondrial matrix. Thus, PDH and KGDH lipoate residues are uniquely powerful sources of metabolic information, reporting mitochondrial matrix energetic status and highly concentrated for convenient statistical “polling” power.

In summary, PDH and KGDH are regulated in mechanistically distinct ways in carcinoma cells. Moreover, these distinct regulatory states respond to the chemical status of enzyme-linked lipoate.

Use of xenobiotic, non-redox-active lipoate analogs to attack tumor-specific mitochondrial metabolism: Lipoate residues in the five complexes discussed above (Figures 1 & 2) are apparently usually synthesized de novo in the mitochondrion directly on the enzymes that use the cofactor; dietary lipoate is thought to rarely or never be used to produce these holoenzymes [5]. Nonetheless, extensive in vitro work demonstrates that the regulatory processes of PDH and KGDH respond to exogenous lipoate in in vitro experiments with purified enzymes [above; reviewed in 6]. Thus, we hypothesized that lipoate analogs might be used to address PDH and/or KGDH regulation, including their re-regulation in carcinoma cells. To explore this hypothesis, we and our collaborators developed and tested a series of lipoate analogs as anti-cancer agents. A subset of these analogs has proven to be capable of potent, selective inhibition of tumor cell mitochondrial metabolism, resulting in tumor cell death.

Specifically, one member of this lipoate analog family, CPI-613, has been especially extensively studied.

CPI-613 inactivates tumor cell PDH selectively, including extensive activation of PDK phosphorylation of the PDH E1 activity [57]. CPI-613 also substantially inhibits KGDH activity through apparent hyper-activation of redox feedback inhibition, again, selectively in tumor cells [56].

CPI-613 is very potent in xenograft tumor growth inhibition assays, wherein the drug displays a very high therapeutic index [57]. Specifically, virtually complete suppression of human tumor xenograft growth can be achieved with doses as low as 0.1 mg/kg [57 and our unpublished results], more than two orders of magnitude lower than doses used to produce modest tumor growth inhibition in corresponding experiments with the parental lipoate compound (above). CPI-613 is well tolerate in these animals up to doses of 10-100mg/kg. Moreover, large-animal toxicology and Phase I clinical trials indicate that CPI-613 is well tolerated in clinically relevant settings. Collectively, these results indicate the possibility of clinical promise.

Phase II clinical trials, including a relatively large study currently underway at Wake Forest University under the direction of Prof. Tim Pardee are producing promising anecdotal indications of response in some relapsed leukemia and lymphoma patients refractory to other currently available therapies [58].

In summary, xenobiotic lipoate analogs appear to have substantial anti-cancer clinical promise. One important goal is to continue to learn more about the carcinoma cell-specific, lipoate-sensitive processes apparently underlying this approach.

## Final summary/overview

The uniquely central role of lipoate in the tumor-specific regulation of mitochondrial metabolism indicates that targeting lipoate-responsive control mechanisms might provide a particularly powerful new strategy for cancer chemotherapy. The evidence reviewed here indicates that the parental, biogenic lipoate molecule is likely to be ineffective in targeting these processes at realistic therapeutic doses, possibly relying primarily or exclusively on the redox activity of this molecule. However, properly designed xenobiotic lipoate analogs apparently have the potential to be successful in this novel chemotherapeutic space.

Specifically, CPI-613 is a non-redox active lipoate analog (Figure 2). CPI-613 is selectively cytotoxic in a large number of tumor cell lines at doses substantially lower than required for parental lipoate cytotoxicity (Figure 2B,D)[57]. In the first hour of treatment with CPI-613, mitochondrial ATP synthesis decreases by 80% to 90% [57]. This rapid and catastrophic reduction of mitochondrial ATP synthesis includes the action of CPI-613 on two key lipoate-containing, dehydrogenase complexes: pyruvate dehydrogenase (PDH) and  $\alpha$ -ketoglutarate dehydrogenase (KGDH) (above). PDH is attacked through its tumor-specific hyper-activation of regulatory kinases and KGDH through tumor-specific hyper-activation of its redox autoregulatory circuitry.

Thus, the proximal mechanism of attack on these two enzymes differs completely, making CPI-613 a “cocktail of one,” simultaneously addressing two apparently independent, essential targets. It will be of great interest to continue to explore the performance of CPI-613 in the clinic.

**EXPERT COMMENTARY:** Lipoic acid (lipoate) occupies a unique position in tumor metabolism.

Enzyme bound lipoate residues generate a strong regulatory signal, reflecting the metabolic status of the mitochondrion in ways that no other molecule can do. Moreover, the machinery responding to this signal is systematically reconfigured in tumor cells. Thus, appropriately designed xenobiotic lipoate analogs can target tumor mitochondrial metabolism with potency and selectivity. As mitochondria play a pivotal role in control of cell death, their targeting results in efficient, selective cancer cell destruction. Thus, lipoate analogs represent a fundamentally new approach to cancer chemotherapy whose potential efficacy is supported by extensive pre-clinical studies and ongoing Phase II clinical trials to date.

**FIVE-YEAR VIEW:** Lipoate analogs have now been subjected to extensive preclinical investigation, validating their possible clinical value and generating a first-generation clinical drug. The relevant mechanistic features of this first-in-class agent set will be subjected to further analysis, potentially opening avenues for further enhancing efficacy and developing next-generation agents. Initial clinical trials are currently ongoing and have produced promising anecdotal indications of efficacy. These trials are expected to be expanded over the next several years with the ultimate goal of arriving at useful new clinical options.

## **KEY ISSUES:**

Robust, perdurant chemotherapeutic suppression of most cancers has remained elusive.

Tumor cells differ from most normal cells in diverse ways, though many of these differences have yet to yield effective therapeutic opportunities.

The cancer community has come to recognize, over the last decade, that tumor metabolism differs substantially and multifariously from the metabolism of most normal cells.

Thus, tumor metabolism has emerged as a new therapeutic target environment whose exceptional promise, however, as yet, remains unrealized.

Lipoic acid (lipoate) is not merely a catalytic coenzyme, but also occupies a unique position in the regulation of metabolism.

Lipoate-dependent regulatory processes are reconfigured in tumor mitochondria, inviting cancer-specific targeting.

Exogenously supplied biogenic lipoate appears to have only limited anti-cancer potency, likely reflecting its capacity to modulate cellular redox potential.

In contrast, appropriately designed xenobiotic lipoate analogs can directly address lipoate-dependent, tumor-specific regulatory targets, resulting in efficient, selective tumor cell death.

Lipoate analogs have been subjected to extensive pre-clinical investigation as chemotherapeutic agents and ongoing Phase II clinical trials to date have produced encouraging anecdotal responses.

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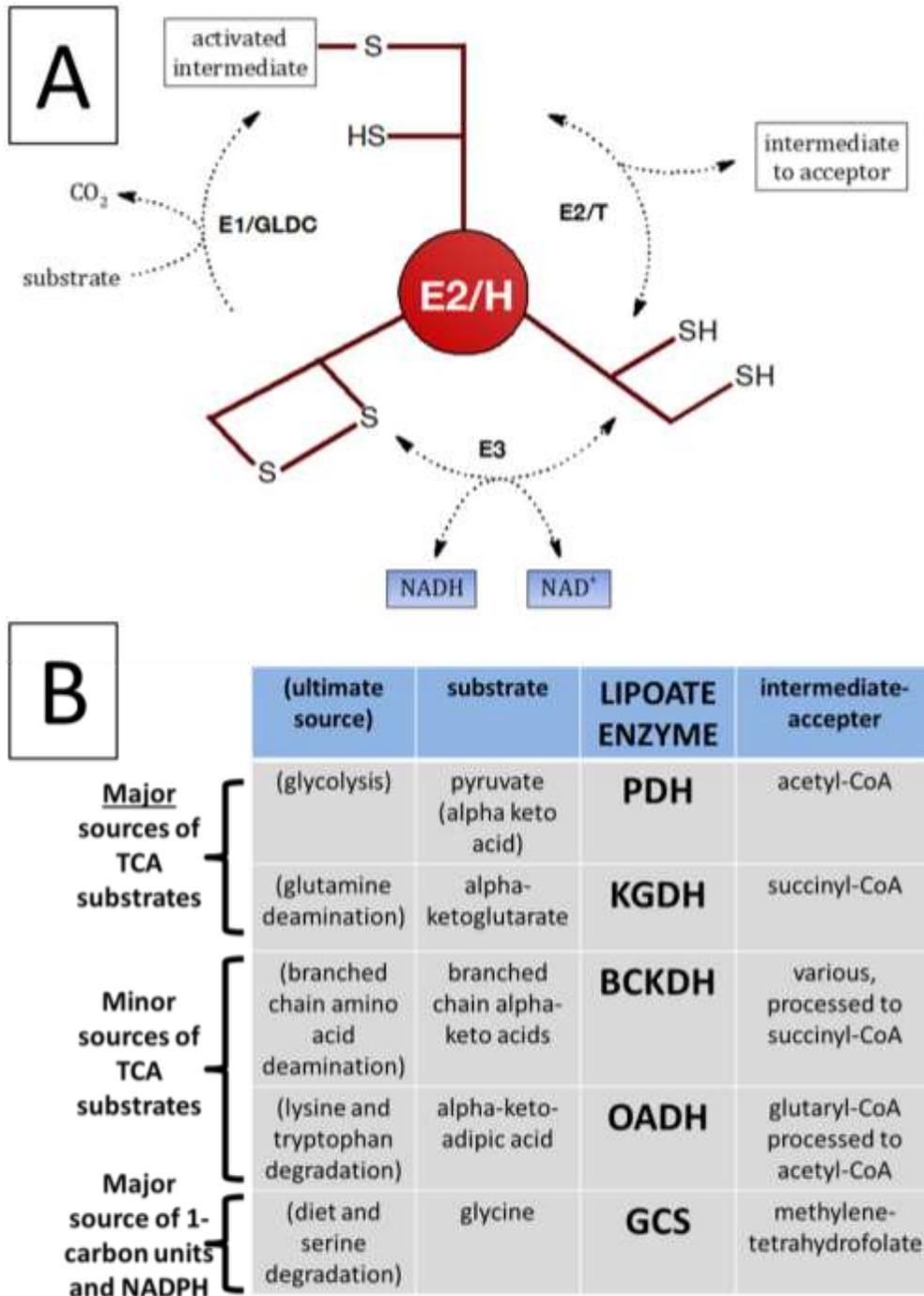
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## FIGURES AND LEGENDS



# Figure 1

## LEGEND TO FIGURE 1: Lipoate-using enzymes in humans

Panel A: Catalytic cycle characteristic of all lipoate multi-enzyme complexes progresses from the 9 o'clock position clockwise. The enzyme catalyzing each step is indicated (dehydrogenase nomenclature/glycine cleavage system, GCS, nomenclature; GLDC stands for glycine decarboxylase; and the E3 dehydrogenase is apparently the same for all five enzymes, though it is traditionally denoted the L subunit in the GCS). The "activated intermediate" is a thioester for each of the dehydrogenases and a methyl-amine thioether for the GCS.

Panel B: List of selected features of each of the five known mammalian lipoate enzymes, all mitochondrial. Note that PDH and KGDH catalyze the entry of glucose- and glutamine-derived carbon, respectively, into the TCA cycle. As a result these two lipoate enzymes govern the large majority of mitochondrial carbon flow in most tumor cells. All the dehydrogenases ultimately introduce carbon into the TCA cycle, while the glycine cleavage system generates a one-carbon unit activated as a tetrahydrofolate (THF) compound together with ammonia as a by-product. This THF-bound carbon can be donated to the cellular one-carbon anabolite pool or be oxidized in the mitochondrion to carbon dioxide with the generation of reducing potential.

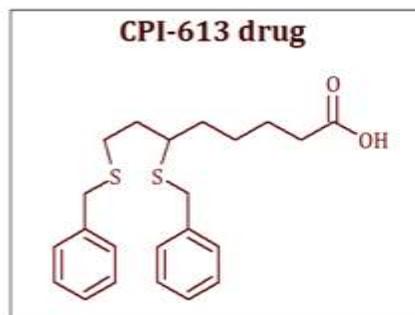
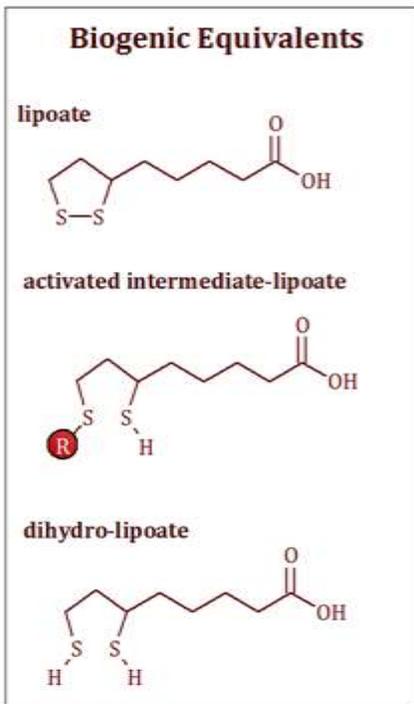
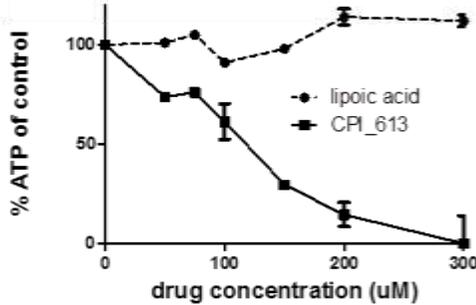
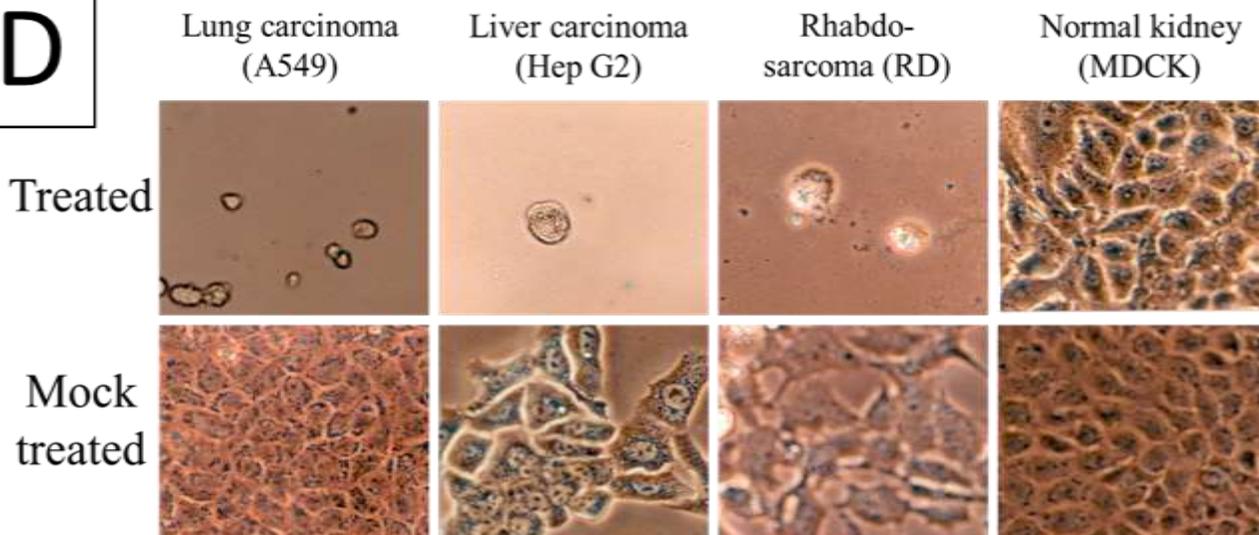
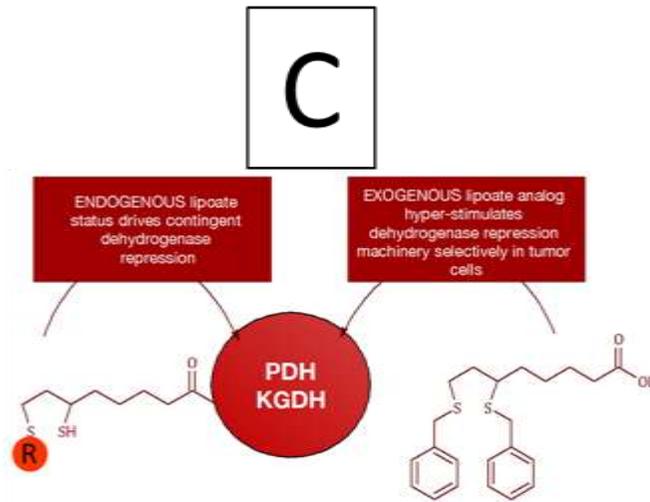
**A****B****D****C**

Figure 2

LEGEND TO FIGURE 2: Lipoate analogs efficiently attack tumor cell metabolism

Panel A shows the generic structures of lipoate intermediates in the functioning of the five mammalian lipoate enzymes (Figure 1) and the structure of CPI-613, a member of the lipoate analog family currently under investigation as anti-cancer drugs.

Panel B shows the killing of tumor cells by CPI-613, but not by the same doses of parental, biogenic lipoate.

Panel C is a cartoon of our current understanding of the mechanism of action of CPI-613 in tumor cells (text).

Panel D shows the efficient, selective killing of tumor cells by a member of the lipoate analog family similar in structure to CPI-613. These images were shot after 24 hours of treatment and show the last few remnants of cancer cells undergoing cell death in the treated panels.