Lipoic acid analogs induce ROS, leading to potent mitochondrial enzyme inhibition, metabolic dysfunction and cell death in tumor cells

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Introduction

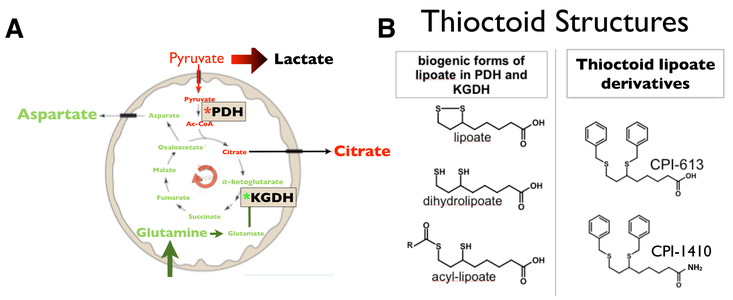

Figure 1: Thioctoid structure. A. Two lipoic acid- containing enzyme complexes, pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (KGDH), stand at major regulatory points governing the flow of carbon through mitochondria. B. Biogenic forms of lipoic acid and the active thioctoid lipoic acid analogs CPI-613 and CPI-1410. CPI-613 and CPI-1410 are designed to mimic lipoate catalytic intermediates which regulate elements of the PDH complex altered in cancer cell metabolism. Note the blocked sulfurs. (Figures 1A modified from [1])

Results

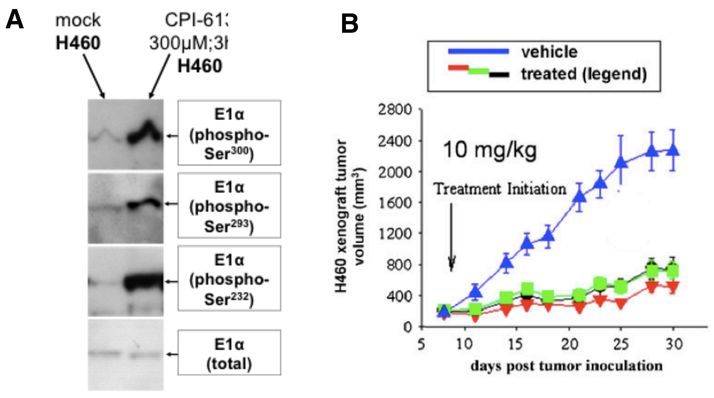

Figure 2 : Thioctoids post-translationally modify tumor cell PDH and inhibit *in vivo* tumor growth.
A. PDH E1α is regulated by the phosphorylation of 3 serine residues. Using phospho-specific antibodies we see thioctoid induced phospho-inactivation of all 3 serines in H460 lung carcinoma cells. B. 10mg/kg CPI-613 at 3 dosing regimens (black, green & red data points) significantly slows tumor cell growth in mouse xenograft models compared to vehicle-treated controls (blue lines). (Figures 2A, B from [2])

Results

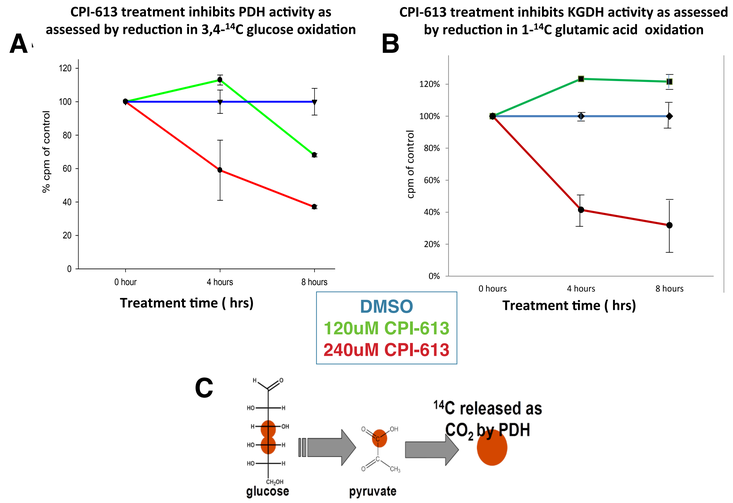

Figure 3: Thioctoids alter mitochondrial carbon flux
A. Mitochondrial carbon flux was monitored using either 3,4-[14C]glucose (A) or 1-[14C] glutamate (B) and quantifying [14C]CO_2 release. Thioctoids potently inhibit the lipoate-containing enzymes PDH and KGDH in a dose- and time-dependent manner. C. Schematic of the reaction quantified in (A). Carbons 3 & 4 of glucose become C-1 of pyruvate after glycolysis and are released as CO_2 after decarboxylation by PDH. C-1 of glutamate is released in an analogous manner by KGDH after conversion to 2-oxoglutarate.

Results

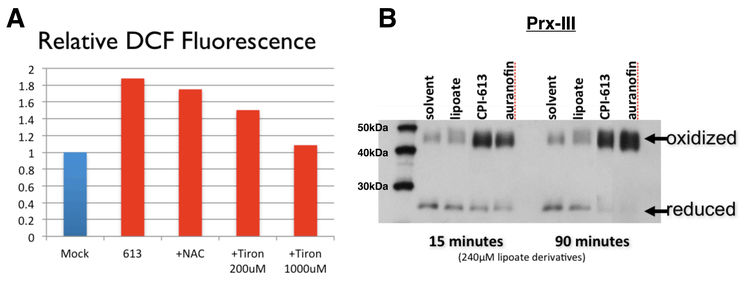

Figure 4: Thioctoids induce mitochondrial-localized ROS.
A. FACS analysis of hydrogen peroxide-induced oxidation of redox-specific, cell permeant dyes showing a large thioctoid-induced increase in cellular ROS levels which can be scavenged by antioxidants (NAC, Tiron). B. Peroxiredoxins are a class of cellular antioxidants that exist in both mitochondrial (Prx-III) and cytosolic (Prx-I) forms. Treatment with active thioctoids results in an increase in the oxidized inactive dimerized form of the mitochondrial Prx-III at early times. The dimerization of the cytosolic Prx-I & -II lag noticeably behind the mitochondrial isoform (data not shown).

Results

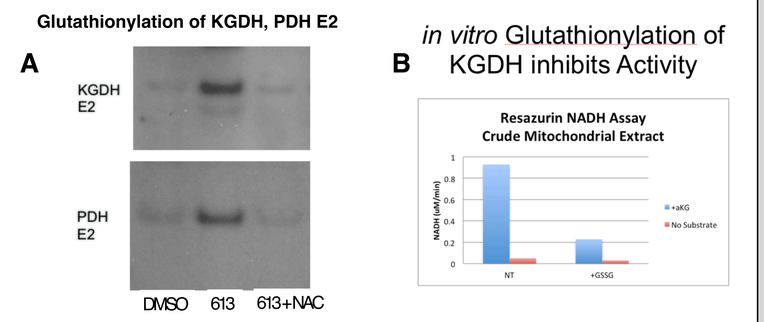

Figure 5: Thioctoids induce glutathionylation of KGDH E2 resulting in enzyme inhibition.
A. H460 lung carcinoma cells were treated for 3 hours with 240uM CPI-613 and glutathionylation status was assayed via the biotin-switch method. The E2 subunits of both KGDH and PDH are detected among glutathionylated proteins in CPI-613-treated samples Glutathionylation is completely inhibited by co-incubation with the antioxidant NAC. B. in vitro KGDH activity was monitored using the NADH-dependent reduction of non-fluorescent resazurin to highly fluorescent resofurin. in vitro glutathionylation of KGDH by glutaredoxin significantly suppresses the activity of the enzyme.

Conclusions

Tumor cell metabolism is emerging as a promising chemotherapeutic target. We have previously shown that thioctoids inhibit PDH via phosphorylation[2]. Here we demonstrate an additional inhibitory effect on the Krebs cycle enzyme KGDH via a ROS-dependent glutathionylation mechanism. Thioctoids appear to target multiple lipoate-containing mitochondrial enzymes and may in fact act as a 'cocktail-of-one,' perturbing tumor cell metabolism at multiple sites. These drugs are currently in human clinical trials and our pre-clinical studies indicate that they may have unique promise.

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References

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2. Zachar Z, Marecek J, Maturo C, Gupta S, Stuart SD, Howell K, Schauble A, Lem J, Piramzadian A, Karnik S, Lee K, Rodriguez R, Shorr R, Bingham PM. "Non-redox-active lipoate derivates disrupt cancer cell mitochondrial metabolism and are potent anticancer agents in vivo." J Mol Med, 2011. 89(11): p. 1137-48.