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# Lactate and malignant tumors: A therapeutic target at the end stage of glycolysis

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# Abstract

Metabolic aberrations in the form of altered flux through key metabolic pathways are primary hallmarks of many malignant tumors. Primarily the result of altered isozyme expression, these adaptations enhance the survival and proliferation of the tumor at the expense of surrounding normal tissue. Consequently, they also expose a unique set of targets for tumor destruction while sparing healthy tissues. Despite this fact, development of drugs to directly target such altered metabolic pathways of malignant tumors has been under-investigated until recently. One such target is the ultimate step of glycolysis, which, as expected, presents itself as a metabolic aberration in most malignant tumors. Termed "aerobic glycolysis" due to abnormal conversion of pyruvic acid to lactic acid even under normoxia, the altered metabolism requires these tumors to rapidly efflux lactic acid to the microenvironment in order to prevent poisoning themselves. Thus, exposed is a prime "choke-point" to target these highly malignant, frequently chemo- and radio-resistant tumors. This review will focus on current outcomes in targeting lactate efflux in such tumors using glioma as a model, an ongoing project in our laboratory for the past half-decade, as well as supporting evidence from recent studies by others on targeting this "tail-end" of glycolysis in other tumor models.

# Keywords

Lactate; MCT; Monocarboxylate transport; Glioma; Malignant tumors

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# Introduction

Our cells harbor a pair of engines that act in tandem to generate and meet the primary energy demands of cell-metabolism. First is the glycolytic pathway, which traps incoming "6-carbon" glucose and converts it to pyruvate, the "3-carbon" form that fuels the cell's ATP turbines, the mitochondria. In the latter, the "3-carbons" are oxidized to CO<sub>2</sub>, the final, fully oxidized "1-carbon" form. Overall, the two processes in series generate the equivalent of 38 ATP units per glucose molecule (Berg et al., 2006). Such is the energetic fate of glucose in healthy tissue under normal physiological conditions.

However, the two engines become badly "misaligned" when tissues become cancerous, with the mitochondrial process being increasingly subverted as the tumor becomes more malignant (Pedersen, 1978; Baggetto, 1992). Long known as the "Warburg effect" (Argiles and Lopez-Soriano, 1990; Gatenby and Gillies, 2004) after its discoverer (Warburg et al., 1930), the tumor cells now increasingly have to rely on reducing the pyruvate to lactate, in order to recycle NADH back to NAD<sup>+</sup> to maintain the metabolic flux via glycolysis. Although energetically unfavorable, this altered scheme most likely helps the tumor to survive and thrive in the hostile microenvironment that it creates during this process, and in fact, metastasize within the host tissue (Gatenby and Gawlinski, 2003; Gatenby et al., 2006; Mathupala et al., 2006; Fantin et al., 2006).

We will discuss the therapeutic possibilities that arise in targeting such malignant tumors as a result of this frequently observed metabolic aberration. In short, lactate, unless absorbed back into the tumor's metabolic cycle, needs to be expelled from the tumor to the microenvironment in order for the tumor to survive by continuing its aberrant glycolytic (glucose to lactate) metabolism. Thus, any strategy to halt the lactate efflux should adversely affect the tumor while sparing the surrounding normal tissues of the same fate.

In addition, the mitochondrial tricarboxylic acid cycle and "offshoots" of the glycolytic pathway, i.e., the pentosephosphate shuttle, account for the major metabolic precursors required for biosynthesis in both normal and tumor tissues (Pedersen, 1978). Thus, any disruption or alteration of the metabolic flux via these two routes in tumors should adversely affect their proliferative capacity and the metastatic potential as well (Fig. 1).

### Monocarboxylate transporters (MCTs): The "pores" that efflux lactate

Decades ago, lactate was thought to be removed from cells primarily via simple transmembrane diffusion as lactic acid, its un-dissociated acid form. However, studies over the last three decades have established the presence of a family of lactate transporters that facilitate the passive transport of lactic acid (and other small organic acids harboring a single carboxyl moiety, i.e., pyruvic acid and butyric acid) across the mammalian plasma membrane (Poole and Halestrap, 1993; Halestrap and Price, 1999; Halestrap and Meredith, 2004). Due to their promiscuity in transporting monocarboxylated metabolites, these transmembrane transporters are referred to as such. Functioning as symporters, they move these organic acids across in their acid form, for example as lactate<sup>-</sup> and H<sup>+</sup>. Recent reports have also indicated the presence of other types of transporters, which utilize Na<sup>+</sup> instead of H<sup>+</sup> (Coady et al., 2004).

Analysis of the human genome has revealed the presence of at least 14 putative members of the H<sup>+</sup>/monocarboxylate transporter family, although to date only four of these, denoted MCT1-4, have been functionally verified to transport H<sup>+</sup> and lactate (Halestrap and Meredith, 2004). However, it should be noted that although the MCTs are responsible for lactate transport, they are not alone in regulating intracellular pH in both normal and tumor tissue. Studies on trans-membrane pH gradients in tumors indicate that the intracellular pH

is, in fact, at physiological levels or even slightly higher compared to the lower pH in the tumor microenvironment (Gillies et al., 1990; Stubbs et al., 1994; Lee and Tannock, 1998; Yamagata et al., 1998; Stubbs et al., 1999).

Thus, the tumors do utilize transmembrane  $Na^+$  gradients (via the sodium-proton exchanger, NHE) (Wahl et al., 2002) or the  $CO_2/HCO_3^-$  gradients (bicarbonate exchanger, BE) (Coss et al., 1997; Owen et al., 1997) in maintaining their transmembrane pH gradients. It remains to be seen whether plasma-membrane proton-pumps (Das et al., 1994; Chi and Pizzo, 2006) are used by the tumors for intracellular pH regulation as well.

# Discussion

In order for MCTs to meet the criteria as suitable therapeutic targets in malignant tumors, they need to differ in their level of expression, the expressed isotypes, or both, between normal and malignant tissues. Our studies on malignant brain tumors have indicated that MCTs 1 and 2 are highly expressed in malignant glioma (Mathupala et al., 2004), while MCT 3 (previously known as type 4) is predominant in normal brain tissue. To date, differential targeting of MCT isoforms has been examined *in vitro* in model glioma (Mathupala et al., 2004) and neuroblastoma (Fang et al., 2006) to test the efficacy of such a strategy.

The function of tumor expressed MCTs can be disrupted via (1) small-molecule inhibitors (Table 1); or (2) by targeting their expression via post-transcriptional gene-silencing strategies; or (3) by targeting accessory membrane proteins including gp70 and CD147 (Kirk et al., 2000; Wilson et al., 2005), that facilitate transmembrane expression of MCTs. Discussed below are the first two methods, which we, and others, have examined to date. Efficacy of the third method, i.e, targeting the "chaperones" that facilitate plasma-membrane expression of MCTs, has not been studied yet.

# Small-interfering RNA (siRNA) and microRNA (miRNA) based isoform-specific targeting of monocarboxylate transporters

Sequence alignment of mRNA from monocarboxylate transporter isoforms 1 to 4 indicates significant sequence similarities among them, making global, isoform specific gene-silencing via classical anti-sense methods difficult. However, with the advent of post-transcriptional gene silencing (PTGS) techniques via siRNA (Fire et al., 1998; Zamore et al., 2000) or miRNA (Lee et al., 1993; Lim et al., 2003), specific targeting of individual MCT isoforms becomes possible.

### siRNA mediated differential targeting of MCTs in glioma causes tumor cell-death

We utilized a plasmid derived siRNA expression strategy to down-regulate MCT 1 and 2 expression in glioblastoma cells *in vitro* to test their effects on intracellular pH, the capacity to efflux lactic acid, and the effect on cell-survival. Efflux of lactate in the targeted cells was reduced by 85% but with only a drop in pH by 0.6 units (a 4-fold increase in intracellular  $H^+$ ) (Mathupala et al., 2004). The latter pH change was likely minimized by the presence of other plasma membrane pH regulators in the tumor cells. However, significant cell-death was observed in the targeted glioma, indicating the applicability of the strategy in a future therapeutic setting. Similar effects have been observed in recent studies targeting neuroblastoma, where the MCT1 was targeted (Fang et al., 2006).

### Targeting the end-stage of glycolysis negatively affects tumor malignancy

Inhibition of the penultimate step in glycolysis, i.e., conversion of pyruvate to lactate via siRNA mediated targeting of lactate dehydrogenase (LDH), was recently reported in murine

mammary tumor cells, both *in vitro* and *in vivo* (Fantin et al., 2006). In the targeted cells, the glycolytic metabolism was repressed, but with an enhancement in mitochondrial respiration. A concomitant reduction in the tumor's proliferative and tumorigenic potential was also observed. These results further support the hypothesis that a highly glycolytic phenotype facilitates the invasive potential of malignant tumors by forcing upon the surrounding normal tissues a truly inhospitable environment.

However, despite the promise of siRNA and miRNA as a therapeutic application for isoform-specific posttranscriptional gene silencing, a primary hurdle remains in introducing these RNA moieties to the target tissue *in vivo* with high efficiency, but with minimum side-effects to the organism. Thus, these issues need to be addressed and overcome prior to these novel and exciting techniques being applicable in a therapeutic setting.

### Use of small-molecule inhibitors of monocarboxylate transporters for localized (tissuespecific) targeting

Being trans-membrane transporters exposed to the extracellular milieu, the MCTs are amenable to targeting by systemic application of small-molecule inhibitors. The "classic" inhibitors of monocarboxylate transporters have been derivatives of cinnamic acid, first identified by Halestrap and co-workers for their effect on isolated mitochondrial pyruvate transport (Halestrap and Denton, 1974), and by Lehninger and co-workers on intact Ehrich ascites tumor (Spencer and Lehninger, 1976). The latter study and studies by others (Wahl et al., 2002; Coss et al., 2003) have indicated the cinnamic acid derivatives to be competitive inhibitors of lactate transport in tumors, with  $\alpha$ -cyano-4-hydroxy cinnamic acid (ACCA), a commonly utilized off-the-shelf chemical used as a matrix during mass spectrometry, as one of the more potent inhibitors of lactate transport, with a K<sub>1</sub> of 0.5 mM.

### Low-affinity inhibitors of lactate transport are effective anti-tumor agents

Studies in our laboratory with  $\alpha$ -cyano-4-hydroxy cinnamic acid (ACCA) for its therapeutic efficacy against malignant glioma indicates it to be an effective cytotoxic and cytostatic agent both *in vitro* and *in vivo*, but at mM (10 mM) concentrations due to its high K<sub>i</sub> against lactate (Colen et al., 2006). Digitonin mediated permeabilization studies on glioma also indicated that ACCA remains extracellular. Thus, the inhibitory effect of ACCA was restricted to the glioma plasma membrane monocarboxylate transport only. Intracellular transporters known to be strongly inhibited by ACCA, i.e., the mitochondrial pyruvate carrier (MPC) (Halestrap and Denton, 1974) were not affected.

Programmed delivery of ACCA to brain in nude rat models also did not present adverse neurological effects or any pathological signatures upon histology (i.e., tissue necrosis at the delivery site) (Colen et al., 2006). Thus, despite the fact that spatially and transiently limited shuttling of lactate occurs between excitatory neurons and their surrounding astrocytes in normal brain (Kasischke et al., 2004; Pellerin and Magistretti, 2004), small-molecule inhibitors of MCTs with high  $K_i$  values against lactate may be optimal for targeting malignant glioma. In fact, when ACCA was delivered to the tumor bed in an orthotopic nude rat glioma model, survival of the animals were enhanced by approximately 2-fold, indicating that the strategy of targeting malignant tumors via inhibition of lactate efflux holds therapeutic applicability without significant side-effects.

ACCA treated glioma were highly radiosensitive. Inhibition of lactate efflux via ACCA in glioma also resulted in key changes to intracellular metabolites including significant reductions in radio-protective metabolites, including reductions in glutathione and taurine (Colen et al., 2006). Alanine and glutamine levels remained relatively unchanged. Thus, the changes in metabolites were most likely due to redirection of glycolytic flux into

mitochondria. These results pave the way for a future combined therapeutic strategy, where the tumors are first exposed to lactate transport inhibitors to enhance their radiosensitivity immediately prior to radiotherapy.

Redirection of metabolic-flux into mitochondria was also observed in the recent study by Fantin and co-workers (Fantin et al., 2006). Here, inhibition of LDH in a mammary tumor model caused redirection of metabolic flux into mitochondria and negatively affected their metastatic potential. Overall, these studies bring forth the manifold potential of metabolic targeting of glioma, with synergistic therapeutic effects including enhanced radiosensitivity and reduced malignancy.

Can these results be replicated in other solid tumors, or via systemic application of smallmolecule MCT inhibitors in general? In a systemic application (in contrast to brain, which is a privileged organ due to the blood-brain barrier) several tissues, most importantly the heart muscle and the liver will be exposed to the inhibitor. Efflux of lactate from cardiac myocytes and influx of the same in hepatocytes for the functioning of the Cori cycle (Berg et al., 2006) are physiologically critical events. Thus, programmed delivery of MCT inhibitors to the tumor bed of a solid tumor will most likely be needed for a successful therapeutic strategy to prevent adverse effects to key organ systems.

In conclusion, the study outcomes presented in this review indicate the efficacy of readily available metabolic inhibitors in targeting the most malignant of tumors, to re-direct its aberrant metabolism towards a more "normal" phenotype. Our studies, and those by others, already indicate that such a strategy reduces the malignancy of the targeted tumor, enhances radio-sensitivity and causes cell-death in the targeted tumor tissue. Thus, a multi-faceted successful outcome may be derived against tumors by further exploring this long-neglected final step of glycolysis as a therapeutic avenue.

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# Abbreviations

MCT	monocarboxylate transporter	
LDH	lactate dehydrogenase	
siRNA	small intefering RNA	
miRNA	microRNA	
CD	cluster of differentiation	

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Mathupala et al.

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Mathupala et al.



#### Fig. 1.

Metabolic fate of pyruvate in normal and tumor tissue. In normal tissue, and under normoxia, pyruvate is primarily directed into mitochondria to facilitate oxidative phosphorylation (A). In malignant tumors (B), pyruvate is primarily reduced to lactate via lactate dehydrogenase (LDH) with concomitant oxidation of NADH to replenish NAD<sup>+</sup> reserves, in order for glycolysis to continue. Lactate is effluxed to the tumor microenvironment via monocarboxylate transporters (MCT), thus subjecting normal tissue in the tumor microenvironment to metabolically hostile conditions, but facilitating the invasion of tumor. Extracellular ACCA inhibits lactate efflux (C) thus inhibiting pyruvate to

Mathupala et al.

lactate reduction. Continued application of ACCA prevents the cycling of NAD<sup>+</sup>, or the functioning of the glycolytic cascade, thus causing metabolic crisis in the tumor cells.

#### Table 1

### Small-molecule inhibitors of MCT-mediated lactate transport

Inhibitor	$K_i$ (mM)	Select References <sup>a</sup>
Derivatives of cinnamic acid	0.5–5	Spencer and Lehninger (1976), Carpenter and Halestrap (1994)
Quercertin	0.05	Volk et al. (1997)
Lonidamine	ND	Rotin and Tannock (1984), Fang et al. (2006)
pCMB, mersalyl <sup>b</sup>	0.007-1.25	Spencer and Lehninger (1976), Carpenter and Halestrap (1994)
Stilbene disulfonates <sup>C</sup>	0.05-1.5	Spencer and Lehninger (1976), Carpenter and Halestrap (1994)
Phloretin <sup>d</sup>	5	Carpenter and Halestrap (1994), Juel and Halestrap (1999)

 $^{a}$ Where available, the references are listed for studies on tumor cells.

 $b_{\rm These}$  broad-spectrum thiol reagents inhibit MCTs by their interaction with CD147 (Wilson et al., 2005).

<sup>c</sup>Also inhibits Cl<sup>-</sup>/HCO<sub>3</sub><sup>-</sup> transport.

 $^{d}$ A broad spectrum, membrane-interactive inhibitor that affects ion-channels and other transporters (e.g., glucose transporter).