Metabolic modulation as a novel cancer treatment

Peter Dromparis, Gopinath Sutendra and Evangelos D. Michelakis, Department of Medicine, University of Alberta, Edmonton, Canada

Correspondence: Evangelos D. Michelakis, University of Alberta, Edmonton, T6G 2B7 Canada. Tel: +1 780-407-1576, fax: +1 780-407-6452, e-mail: em2@ualberta.ca

Abstract
The Warburg effect refers to the cancer’s metabolic shift from mitochondrial oxidation to anaerobic glycolysis, even when oxygen is present. Although this metabolic anomaly was long believed to be due to irreversible mitochondrial damage, it now appears mitochondria in cancer are actively suppressed. Since mitochondria are extensively integrated into the metabolic, signaling and apoptotic biology of the cell, mitochondrial suppression in combination with glycolysis-driven metabolism allows cancer to masterfully inhibit intrinsic cell death mechanisms and promote rapid proliferation even in sub-optimal conditions. In this review, we analyze cancer’s metabolic strategy and describe how active mitochondrial suppression is compatible with both the genetic and evolutionary models of cancer. Furthermore, we discuss several strategies for mitochondrial activation and review current pre-clinical and clinical studies of agents showing promise in effectively and selectively targeting cancer.

Keywords: cancer, metabolic, apoptosis, dichloroacetate, glycolysis

Introduction
We currently perceive cancer as an accumulation of numerous chromosomal, genetic and biochemical abnormalities that result in dysfunctional cells. However, if so extensively damaged, how does cancer escape death, even flourish, in hypoxic and acidic conditions? Perhaps cancer should be viewed as the “healthiest” of cells, developing exquisite mechanisms to suppress cell death (which is mostly regulated by mitochondria) and survive in otherwise uninhabitable microenvironments, essentially achieving what medicine strives for—immortality. One way that cancer could achieve this is by developing strategies to effectively suppress mitochondrial function. Here, we summarize cancer’s metabolic strategies to evade death and discuss potentially effective and selective mitochondria-targeting therapies that induce apoptosis in cancerous but not non-cancerous tissues.

Mitochondria: a central role in cellular biology
Mitochondria produce energy through the oxidation of carbohydrates and lipids. In glucose oxidation (GO), pyruvate is de-carboxylated to acetyl-CoA by the gate-keeping mitochondrial enzyme pyruvate dehydrogenase (PDH). In fatty acid oxidation (FAO), fatty acids enter the mitochondria through carnitine palmitoyl-transferase-1 (CPT-1) and are also converted to acetyl-CoA (Fig. 1). The Krebs’ cycle extracts electrons derived from acetyl-CoA and feeds
Mitochondria are major regulators of apoptosis. Upon opening of the mitochondrial transition pore (MTP), pro-apoptotic mediators are released into the cytoplasm and ignite the apoptotic process. MTP is a mega-channel that spans the mitochondrial membrane and is both redox- and voltage-sensitive, thus regulated in part by $\Delta \Psi_m$ (Fig. 1) [1]. While $\Delta \Psi_m$ depolarization promotes MTP opening, hyperpolarization increases its opening threshold, establishing an apoptosis-resistant state. Thus, $\Delta \Psi_m$ could also be a surrogate for apoptosis resistance. Furthermore, since $\Delta \Psi_m$ depends on metabolism, fuel supply and apoptosis are intrinsically linked. As discussed below, most solid cancers are characterized by hyperpolarized mitochondria compared to non-cancerous tissues, pointing to mitochondria’s critical role in the well-known apoptosis resistance in cancer.

Mitochondria are extensively integrated into cell signaling. In addition to $H^+$ extrusion, the ETC also generates superoxide (mitochondria-derived reactive oxygen species – mROS). Unstable mROS, like superoxide, can be dismutated into more stable $mROS$ and leave the mitochondria to regulate redox-sensitive targets like the voltage-gated potassium channels (Kv) in the plasma membrane. As mitochondria are important $O_2$ sensors, this axis (ETC-mROS-Kv channels) is the basis of hypoxic pulmonary vasoconstriction. mROS also regulate other redox-sensitive targets like the transcription factors p53 [2] and hypoxia inducible factor-1α (HIF1α) [3], important in both vascular diseases and cancer (Fig. 1).

Mitochondria may also signal by releasing metabolic substrates. Alpha-ketoglutarate ($\alpha$KG) is a Krebs’ cycle intermediate that, once in the cytosol, acts as a co-factor for prolyl-hydroxylases which degrade HIF1α [3]. In other words, this critical transcription factor that drives angiogenesis in both cancer and vascular diseases, is regulated by at least two mitochondria “signals”, mROS and $\alpha$KG, both of which are linked to fuel processing and mitochondrial respiration.

Mitochondria can also sequester calcium. Being the most negatively charged organelles, they function as Ca$^{++}$ sinks, in a $\Delta \Psi_m$-dependent manner. Among the myriad of Ca$^{++}$-dependent signaling processes are transcription factors that are integral in both vascular disease and cancer, like the nuclear factor of activated T-cells (NFAT) [3].

Therefore, suppression of mitochondrial function has the potential to influence a myriad of cellular processes critical in cancer biology. Suppressed
mitochondria would increase MTP opening thresholds, suppress mROS, inhibit Kv-channels, and activate HIF1α and NFAT, all promoting a proliferative and anti-apoptotic state.

Mitochondria as integrators of early genetic and environmental signals in cancer

A number of common molecular abnormalities described in cancer have direct metabolic and mitochondrial effects that result in suppressed oxidative phosphorylation and mitochondrial function. For example, p53 loss-of-function, cancer’s most common genetic anomaly, strongly promotes a glycolytic phenotype by upregulating glycolytic enzymes including phosphoglycerate mutase and the rate-limiting enzyme hexokinase (HK) [4]. p53 also regulates Ts53-induced glycolysis and apoptosis regulator (TIGAR), an enzyme with fructose-2,6-bisphosphatase activity [2]. When suppressed, fructose-2,6-bisphosphate accumulates and potently simulates the rate-limiting glycolytic enzyme phosphofructokinase-1. Moreover p53 regulates the expression of the ETC protein cytochrome-c oxidase and suppresses the expression of glucose transporters (GLUTs) [2]. Thus, as a result of p53 loss-of-function, ATP supply is maintained by elevated cytoplasmic glycolysis and glucose uptake, compatible with the increased 18-fluorodeoxyglucose positron emission tomography (PET) signal in most tumors compared to non-cancerous tissues.

Similarly, activation of Akt (or PTEN loss-of-function) not only enhances the activity of rate limiting glycolytic enzymes but also translocates GLUTs to the cell membrane. c-myc upregulates nearly all glycolytic enzymes including HK [4, 5]. Intriguingly, in addition to regulating carbohydrate metabolism, several cytoplasmic glycolytic enzymes directly suppress apoptosis [5] or even modulate mitochondrial function. For example, activated HK translocates to the outer mitochondrial membrane and binds the voltage-dependent anion channel (VDAC) [6]. This prevents anion efflux contributing to the ΔΨm hyperpolarization that characterizes most cancers [7] and prevents MTP opening [6].

Mitochondrial enzyme mutations also impair mitochondrial function and promote a glycolytic phenotype. The Krebs’ cycle enzymes succinate dehydrogenase (SDH) [8], fumarate hydratase (FH) [9] and isocitrate dehydrogenase (IDH) [10, 11] are all associated with cancers like renal cell carcinoma, paragangliomas, or glioblastomas.

Thus, mitochondria appear to integrate a large number of diverse genetic signals that all result in a common phenotype (i.e., hyperpolarized mitochondria with suppressed function, upregulated glycolysis and secondary signaling consequences) promoting proliferation and suppressing apoptosis. The evolving metabolic theory of cancer that suggests that the mitochondrial and metabolic remodeling promotes a pro-proliferative and apoptosis-resistant state is compatible with the genetic theory of cancer that has dominated the field for forty years but has failed to deliver effective and selective cancer therapies (except few and isolated examples, such as imatinib mesylate).

Actively suppressed mitochondria are also compatible with the evolutionary model of cancer, which suggests that early carcinogenesis is hypoxia-driven [12]. Here, decreased oxygen suppresses respiration and limits proton influx through ATP synthase. This would result in mitochondrial hyperpolarization and subsequent mitochondria-dependent metabolic signaling and apoptosis suppression. HIF1α facilitates a shift from mitochondrial oxidation to glycolysis, which ensures ATP production even in sustained hypoxia [12]. In addition to upregulating GLUTs, HIF1α upregulates pyruvate dehydrogenase kinase (PDK), the enzyme that tonically inhibits PDH, limiting pyruvate flux into the mitochondria (Fig. 1). When angiogenesis occurs and tumor oxygen delivery is restored, the upregulated PDK maintains mitochondrial suppression, and at this point, loss of secondary mitochondrial signals (mROS, αKG) sustains HIF1α even in the absence of hypoxia, maintaining a glycolytic state.

Mitochondrial suppression in cancer may have pro-proliferative effects in addition to the apoptosis inhibition and HIF1α driven angiogenesis. Metabolites that are now not oxidized in the mitochondria are shifted toward biosynthetic pathways important for rapidly proliferating cells [13]. For instance, glucose-6-phosphate (the product of HK) can be shunted into the pentose phosphate pathway (PPP) whereby ribulose-5-phosphate is produced for nucleotide synthesis [13]. Thus the beneficial effects of an overall mitochondrial suppression in cancer may extend to multiple levels.
Mitochondrial activators in cancer

Not surprisingly, therapies targeting single genetic or molecular abnormalities are ineffective in most cancers, which are commonly heterogeneous in nature. For example, there is significant diversity in glioblastoma multiform (GBM), where a given tumor includes multiple abnormalities and therefore is unlikely to respond to single target strategies [14]. A common feature among all GBM (and most solid tumor) cells is metabolic remodeling. In the 1920s, Warburg suggested that this feature of most cancers is caused by abnormal mitochondria. However, his theory was dismissed and most considered mitochondria inhibition as a secondary effect, possibly a result of oxidative damage.

DCA and preclinical studies

Recently, we showed that cancer cells treated with dichloroacetate (DCA), a PDK inhibitor (and thus a PDH activator), acutely increased GO [15]. Because GO occurs exclusively in the mitochondria, these data suggested that cancer mitochondria are perhaps functionally and reversibly suppressed, not permanently damaged, providing strong rationale for the development of similar drugs as novel cancer therapies. We first published the preclinical effects of DCA in cancer in 2007. We found that non-small cell lung, breast and GBM cancer cells had hyperpolarized ΔΨm compared to non-cancerous cells and that DCA rapidly depolarized mitochondria to normal levels, suggesting restoration of mitochondrial function [15]. In keeping with this, DCA decreased the glycolysis to GO ratio by enhancing GO and lowering lactate production. DCA induced mitochondria-dependent apoptosis and reduced proliferation both in vitro and in vivo [15]. DCA-treated cancer cells had increased mROS, activated Kv-channels and inhibited NFAT. Importantly, DCA did not affect mitochondria and their downstream targets in non-cancerous cells. DCA also decreased tumor size in a xenotransplant model (Fig. 2). Since then, many independent studies have confirmed our findings (reviewed in [16]) in colon [17], prostate [18], endometrial [19] or metastatic breast cancer [20]. In vivo, DCA was effective in attenuating aggressive metastatic breast cancer [20] (Fig. 2). In another study, DCA was chemically linked to cisplatin, a commonly used cancer drug that induces DNA cross-linking [21]. This new molecule, mitaplatin, was cancer selective, inducing mitochondrial-dependent apoptosis and reversing the apoptosis resistance that limits cisplatin’s effectiveness.

DCA and early clinical experience

DCA has been used clinically for decades to symptomatically treat congenital mitochondrial diseases including PDH deficiencies and their resulting lactic acidosis. This, along with promising pre-clinical findings provided the rationale for early phase clinical trials in cancer. The first such study was recently published examining DCA’s effects in GBM, a highly vascular and deadly brain cancer [22]. Examination of 49 freshly excised tumors showed mitochondrial hyperpolarization compared to non-cancerous brain tissue, which was reversed with acute DCA, supporting reversible mitochondrial inactivation in this tumor. The tumors showed high PDK2 expression, the most DCA-sensitive PDK isoform [23] (Fig. 3a). DCA was orally administered to five (previously treated) patients at a dose of 6.25mg/kg bid giving plasma concentrations of 0.266-0.626mM after at least three months, well within the range for PDK2 inhibition (Kc=0.2mM [23]). Importantly, none of the patients developed hematologic, hepatic, renal or cardiac toxicity. Peripheral neuropathy was the only apparent toxicity, which
was reversed at lower doses. This study’s major strength was comparing tumor tissue obtained before and after DCA therapy. Post-DCA, tumor tissues exhibited higher PDH activity, confirming PDK2 inhibition in vivo. Further examination revealed p53 activation, HIF1α inactivation and decreased vascularity. Moreover, the tumors were less proliferative, more apoptotic (Fig. 3a) and some, but not all, showed evidence of regression or stability (Fig. 3b) [22]. Even more exciting was the apparent effects on putative GBM stem cells (GBM-SC; CD133+/Nestin+), which are the most apoptosis-resistant cells and the cause of recurrence after remission. In line with this, the GBM-SC had the highest ΔΨm within the tumor in vivo. DCA induced apoptosis in these cells in vitro (Fig. 3c) and in vivo, suggesting that this traditionally resilient population is also metabolically vulnerable [22].

**Lactate dehydrogenase**

LDH-A, a downstream target of HIF1α[3], converts pyruvate into lactate, restoring NAD+ to sustain glycolysis and preventing pyruvate oxidation. Inhibition of LDH-A may restore mitochondrial function by promoting mitochondrial pyruvate metabolism, in a sense mimicking DCA (Fig. 1). LDH-A inhibition decreases lactate, depolarizes mitochondria, increases respiration and
decreases proliferation in cancer cells [24, 25]. Animals injected with LDH-A-knockdown cancer cells had smaller tumors and improved survival [24], and inhibition of LDH-A reduced tumor volumes in lymphoma and pancreatic xenotransplant models without kidney, liver or blood toxicity [25]. These promising preclinical results and the relative selectivity for cancer suggest LDH-A inhibitors may be a viable therapeutic intervention.

Pyruvate kinase M2 (PKM2)

Conversion of phosphoenol-pyruvate to pyruvate occurs as the final rate-limiting glycolytic step. This reaction is catalyzed by pyruvate kinase, which exists in high (PKM1) and low activity (PKM2) isoforms. The recent identification of PKM2 has generated excitement since this isoform is selective to rapidly proliferating cells [13]. Although it appears paradoxical, this low-activity isoform may actually promote proliferation by reducing ATP production (preventing allosteric inhibition of prior glycolytic reactions) and shunting metabolites into biosynthetic pathways [13, 26]. PKM2 would also reduce mitochondrial pyruvate influx (Fig. 1). Thus, targeting PKM2 not only reduces biosynthetic processes, but may also promote GO, similar to DCA. Indeed, inhibition of PKM2 increases GO, similar to DCA. The PKM2 data are compatible with the PDH/DCA work and the idea of an overall suppression of mitochondrial function in cancer.

Fatty acid metabolism inhibitors

Cancer’s highly proliferative nature creates a large demand for fatty acids for membrane synthesis. Rather than extracellular sources, cancer obtains fatty acids via de novo synthesis from the Krebs’ cycle intermediate citrate [28]. Citrate is upstream of many Krebs’ cycle enzyme mutations commonly associated with cancer as described above. Mutations in these enzymes would lead to accumulation of upstream metabolites (i.e., citrate) for biosynthetic purposes (Fig. 1). Inhibition of several fatty acid biosynthetic steps has shown efficacy in preclinical cancer models [29, 30]. Intriguingly, fatty acid oxidation (FAO) inhibition also inhibits cancer growth [31], as it indirectly promotes GO through the Randle cycle (i.e. FAO-generated acetyl-CoA inhibits PDH) [32]. In other words, this strategy is similar to DCA in terms of “refueling” mitochondria with pyruvate and reversing the mitochondrial suppression and the secondary upregulation of glycolysis.

Glycolysis inhibitors

Cancer’s glycolytic environment provides sufficient energy and building blocks for proliferation while simultaneously suppressing apoptosis. Although direct glycolysis inhibition seems logical, this strategy rapidly (and predictably) depletes ATP causing ATP-independent (necrotic) cell death, which is likely to damage non-cancerous tissues as well. Inhibiting glycolysis may not be selective since non-cancerous tissues (i.e., skeletal muscle, brain, etc.) also rely on glycolysis. In fact, several recent clinical trials of glycolysis inhibitors have not been successfully completed. Clinical trials for 2-deoxyglucose (Hk inhibitor) for prostate cancer (NCT00633087) and intracranial neoplasms (NCT00247403) have been suspended. Phase II/III trials for the Hk-inhibitor lonidamine in benign prostate hyperplasia have also been terminated (NCT00435448, NCT00237536). Another Hk inhibitor, 3-bromopyruvate, which pre-clinical work suggests induces necrosis in cancer, has also demonstrated significant toxicities in animal models at doses only slightly higher than therapeutic doses[33]. In other words, the non-selectivity of these drugs may limit their translational potential. In summary, the metabolic modulators that improve coupling between glycolysis and GO, “refueling” mitochondria and “normalizing” remodeled metabolism should not be confused with glycolysis inhibitors, which rather than normalize metabolism, cause energy starvation (Fig. 1).

Conclusions

Revisiting Warburg’s original hypothesis that abnormal metabolism causes cancer has provided newfound optimism. Once thought to be a secondary result of upstream abnormalities, it now appears that cancer’s metabolic remodeling is integral for survival and exposes many therapeutic targets. Various strategies that restore mitochondrial metabolism have shown selectivity and efficacy in pre-clinical studies and need to efficiently be moved into clinical trials.

References


