Cancer and inhibition of fatty acid oxidation

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Abstract

Recently, a "metabolic transformation" property with increased glycolytic rates (Warburg effect) was described for cancer cells. It has been shown that there is an active interaction between mutations of crucial enzymes involved in metabolic pathways and of oncogenes, whose effector proteins also exert metabolic functions. Interestingly, because many of these metabolic changes are confined to the mutant clones, targeted therapy appears very promising in terms of both safety and efficacy. Pharmacological inhibition of glycolysis by dichloroacetate (DCA) significantly inhibits proliferation of cancer cells. However, because of the pharmacokinetic characteristics, the plasma concentration levels and, therefore, toxic effects (i.e., hepatotoxicity and neurotoxicity) of DCA are difficult to predict. Similar metabolic modulation and antiproliferative effects can be obtained through inhibition of fatty acid oxidation. Such agents have been safely used in other clinical conditions such as heart disease and, as such, deserve further testing in cancer therapy.

Keywords: cancer, Warburg effect, metabolic modulation therapy, fatty acid oxidation

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Cancer continues to ferociously strike humanity. This has led to a major effort to develop new therapies to treat cancers. While major advances have been made in the treatment of this debilitating disease, it still remains a major cause of mortality in our society. As a result, new therapies to treat and prevent cancer are being sought.

Over the past decades, cancer studies have mainly focused on identifying gene and protein networks involved in the regulation of cell growth, differentiation, and death. However, although the genetic mutations may be specific to the tumor, the downstream effectors are also found in normal tissues, therefore resulting in a narrow range of effective and safe molecular targets. Recently, a "metabolic transformation" property, amenable to metabolic modulation therapy, was described for cancer cells. Importantly, because many of these metabolic changes are confined to the mutant clones, targeted therapy appears very promising in terms of both safety and efficacy.

Aerobic glycolysis (Warburg effect) [1] constitutes a common feature of cancer cells and, as such, appears to confer resistance to cell death. Importantly, increases in glycolytic rates in cancer cells indirectly reflect also a decrease in the "mitochondrial energy production state" of cancer cells. Similar to normal growth, apoptosis is an encoded cellular phase that implies adequate mitochondrial energy production and signaling, which ultimately causes the release

of pro-apoptotic factors. Alterations in cellular metabolic pathways appear therefore to play an important role in conferring cancer cells their distinctive "immortality status". In accordance with these observations, genetic analyses have shown that cancer cells displaying these features have mutations of crucial enzymes involved in metabolic pathways (i.e., phosphoinositol-3-kinase [PI3K], Akt, AMP-activated protein kinase [AMPK] and its upstream kinase LKB1, etc.) [2]. In addition, oncogenes whose effector proteins also exert metabolic functions (i.e., p53 target TP53-induced glycolysis and apoptosis regulator [TIGAR], anti-apoptotic protein bcl-2, etc.) also show mutations [3]. Such findings indicate that metabolic and apoptotic pathways are more than two associated phenomenon and therefore represent the scientific basis for considering metabolic modulation as a treatment strategy for cancer.

With regard to metabolic transformation, the key events that cause cancer cells to display the characteristic altered metabolic features are the "pseudo-hypoxic" mitochondrial oxygen sensing with abnormal activation of the hypoxia-induced factor 1-alpha (HIF1-alpha). Increased HIF1-alpha expression activates a series of glycolytic genes, and also suppresses the glucose oxidation activity of the mitochondria by transactivating the pyruvate dehydrogenase (PDH) kinase enzyme (PDK), which phosphorylates and inhibits the PDH complex [4]. PDH is a key enzyme in controlling the rate of glucose oxidative metabolism in that it catalyzes the irreversible oxidation of pyruvate, yielding acetyl-CoA and CO_2 and, therefore its inhibition creates a glycolytic shift of glucose metabolism (Fig. 1). This phenomenon is associated with other mitochondrial-metabolic abnormalities that include: mitochondrial hyperpolarization, decreased superoxide dismutase-2 (SOD2) with reduced production of reactive oxygen species (ROS), and decreased Kv (voltage gated) 1.5 expression. Loss of Kv 1.5 depolarizes the membrane and elevates cytosolic K^+ and Ca^{2+}, leading to Ca^{2+}-calcineurin dependent activation of the proliferative transcription factor (NFAT) and inhibition of caspases through elevation of cytosolic K+.

The relevance of these changes in the coupling of glycolysis to glucose oxidation on cellular proliferation capacity is confirmed by the inhibitory effects on cell proliferation observed through pharmacological inhibition of PDK. Dichloroacetate (DCA) is the most frequently tested pharmacological agent for this purpose (Fig. 2). Besides metabolic changes, DCA restores mitochondrial ROS production capacity, releases

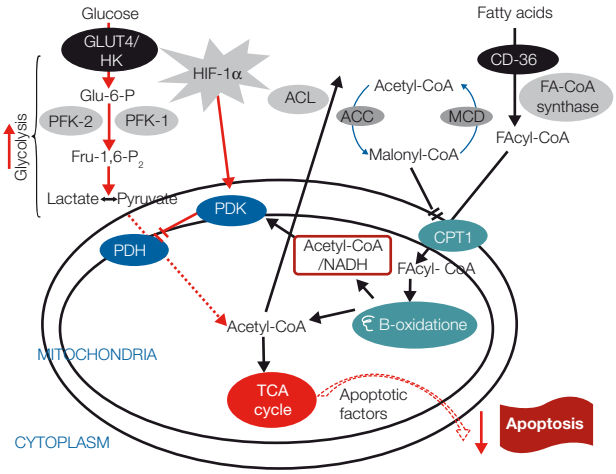

Fig. 1 *Metabolic transformation of cancer cells. Hypoxia induced factor-1α (HIF-1α) causes a pathological activation of pyruvate dehydrogenase (PDH) kinase (PDK), which in turn inhibits PDH activity, therefore limiting oxidation of pyruvate, which is shifted towards lactate production. A reduced glucose oxidation-derived acetyl-CoA has a crucial role in the normal signaling of mitochondria for the release of apoptotic factors.* ACC *acetyl-CoA carboxylase,* ACL *ATP-citrate lyase,* CPT1 *carnitine palmtoyltransferase 1,* GLUT4 *glucose transporter-4,* Glu-6-P *glucose-6-phosphate,* HK *hexokinase,* MCD *malonyl-CoA decarboxylase,* PFK *phospho fructo kinase,* TCA *tricarboxylic acid*

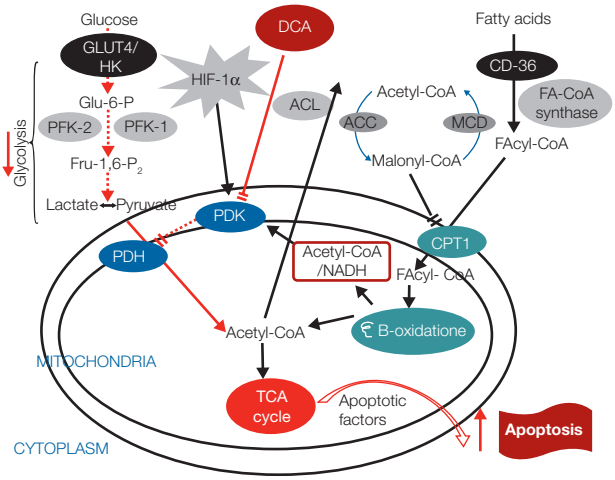

Fig. 2 *Treatment of cancer cells with dichloroacetate (DCA). DCA inhibits pyruvate dehydrogenase (PDH) kinase (PDK), thereby causing a relief of PDH, which catalyzes the decarboxylation of pyruvate to acetyl-CoA. In this way there is a decrease in glycolysis and an increase in apoptotic capacity.* ACC *acetyl-CoA carboxylase,* ACL *ATP-citrate lyase,* CPT1 *carnitine palmtoyltransferase 1,* GLUT4 *transporter-4,* Glu-6-P *glucose-6-phosphate,* HK *hexokinase,* HIF-1α *hypoxia induced factor-1α,* MCD *malonyl-CoA decarboxylase,* PFK *phospho fructo kinase,* TCA *tricarboxylic acid*

pro-apototic factors [5] and, ultimately, decreases cell survival and increases apoptosis rates [4]. However, despite these promising cytological effects, the potential use of DCA in cancer patients is regarded with some degree of skepticism. In fact, when administered for other medical conditions (such as lactic acidosis, sepsis, burns, etc.), DCA has been reported to cause important adverse effects such as hepatotoxicity and neurotoxicity (although to some degree, these toxic effects are reversible with short-term administration) [6]. Such effects would be particularly relevant in cancer patients who are frequently on concomitant neurotoxic chemotherapy. Moreover, since multiple administrations of DCA leads to inhibition of hepatic enzymes responsible for its own metabolism, plasma concentration levels and therefore toxic effects of the drug are difficult to predict [7].

DCA is not the only available agent to stimulate PDH activity. The effects of DCA to increase glucose oxidation rates can be achieved by inhibiting mitochondrial fatty acid oxidation rates [8]. The reciprocal relationship between glucose and fatty acid metabolism is part of a phenomenon known as the "Randle cycle." Since glucose oxidation and fatty acid oxidation both produce mitochondrial acetyl-CoA, the rate of each other's activity has a direct reciprocal effect on the rates of others pathway. Therefore stimulation of glucose oxidation through direct inhibition of PDK activity can similarly be obtained by inhibiting fatty acid oxidation. This has been the rationale for the development and use of fatty acid oxidation inhibitors in other conditions such heart disease. However, at this point one may argue: how can this approach be transferred to cancer cells when the latter have been defined by relying on glycolysis rather than oxidative metabolism?

Nevertheless, although still recognizing glycolysis as the main source for energy production of cancer cells, increasing the coupling of glycolysis to glucose oxidation by switching existing mitochondrial oxidative metabolism from fatty acids to glucose, rather than a reduced overall oxidative capacity of the mitochondria, has been proposed [9]. Therefore, similar to cardiac disease, stimulation of glucose oxidation through inhibition of fatty acid oxidation may be a reasonable approach for cancer therapy (Fig. 3). In support of this concept, fatty acid inhibitors such as etomoxir and ranolazine have been shown to have beneficial effects in leukemia cells [10]. Trimetazidine, another

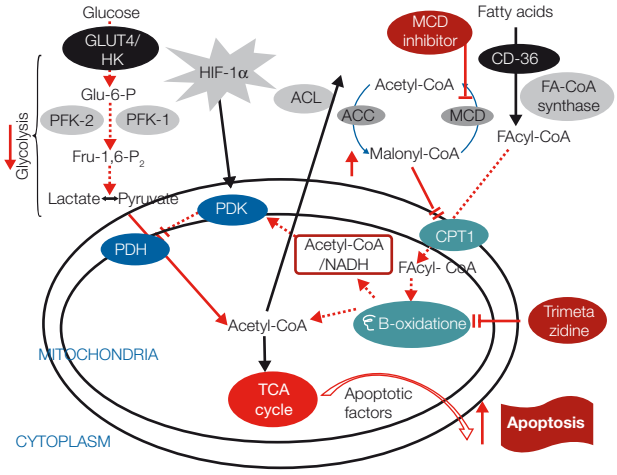

Fig. 3 *Treatment of cancer cells with fatty acid oxidation inhibitory agents. Malonyl-CoA (an endogenous inhibitor of CPT1) levels depend on acetyl-CoA carboxylase (ACC) (involved in its production) and malonyl-CoA decarboxylase (MCD) (involved in its degradation) activities. Inhibition of MCD causes an increase in malonyl-CoA, thereby inhibiting the entry of fatty acyl-CoA moieties for oxidation into mitochondria. Trimetazidine exerts its functions more distally, inhibiting one of the enzymes of the beta-oxidative process. The common effect is a reduced pyruvate dehydrogenase (PDH) kinase (PDK), PDK activity and therefore an enhanced glucose oxidative capacity.* ACL *ATP-citrate lyase,* CPT1 *carnitine palmtoyltransferase 1,* GLUT4 *glucose transporter-4,* Glu-6-P *glucose-6-phosphate,* HK *hexokinase,* HIF-1α *hypoxia induced factor-1α,* PFK *phospho fructo kinase,* TCA *tricarboxylic acid*

fatty acid oxidation inhibitor, was recently shown to dose dependently induce cancer cell apoptosis in experimental conditions [11]. Similarly, Zhou et al showed that increasing malonyl-CoA levels (a key enzyme that inhibits the transfer of fatty acids for oxidation into the mitochondria) through inhibition of malonyl-CoA decarboxylase (MCD), can decrease proliferation of human breast cancer cells [12]. However, the pro-apoptotic effects in the latter two studies were mainly attributed to modifications in fatty acid synthesis (FA) that occurred in addition to trimetazidine and the MCD inhibitor. Indeed, besides increased glycolysis, cancer cells have been also shown to display increased *de novo* FA synthesis [13] with high levels of the lipogenic enzymes ATP-citrate lyase (ACL), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FAS). However, modulation of FA synthesis has yielded contrasting results. Likewise, in the study by Andela et al [11], trimetazidine was tested in addition to a peroxisome proliferator receptor gamma (PPARγ) agonist, which, among other effects, causes an increase in FA synthesis. On the contrary, in the study by Zhou et al,

the MCD inhibitor was tested with cerulin, a FAS inhibitor [12]. These contrasting results in the modulation of FA synthesis may, to some degree, reflect the tissue-specific (in this case cancer cell type) expression of enzymes involved in the regulation of FA. However, both studies showed clear reduction of cellular proliferative capacity, in this way highlighting the potential relevance of fatty acid inhibition and therefore, as mentioned, relief of glucose oxidation, as a therapeutic approach to treat cancer. This is ultimately in line with the beneficial results obtained from direct stimulation of glucose oxidation with DCA. Importantly, the increased apoptotic rates are confined only to cancer and not to normal control cellular lines. Moreover, trimetazidine has been widely tested in human clinical trials, and shows a high safety profile and no major adverse events. In this particular setting, the beneficial results were observed in experimental conditions and do not represent the current indication of the product (Vastarel® MR), which is an established therapy for the management of patients with myocardial ischemia. However, trimetazidine has been shown to have cardioprotective effects on cancer patients with anthracycline-induced acute cardiotoxity [14, 15]. Alternatively, MCD inhibitors, another drug class that inhibit fatty acid oxidation, can be further studied in cancer setting.

In conclusion, altering glucose and fatty acid oxidation in cancer cells provides a potential new approach to treat cancer. The appealing potential of controlling cell proliferation by metabolic modulation therapy has engendered new hopes on the horizon. Increasing glucose oxidation rates, through either a direct induction of glucose oxidation or, a reduction in the fatty acid oxidation rates appears to be a logical approach to treat cancer. DCA, the main direct glucose oxidation stimulator available, has shown to have adverse effects that may be particularly relevant in patients who receive concomitant chemotherapeutic agents. On the other hand, inhibitory agents of fatty acid oxidation appear to have a safer therapeutic profile and may therefore represent a valid alternative to DCA. Given the quickly fatal natural history of the disease and the availability of these agents, efforts to translate these findings in clinical practice are urgently needed. •

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