Review

Cancer cell metabolism and mitochondria: Nutrient plasticity for TCA cycle fueling

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ABSTRACT

Warburg's hypothesis that cancer cells take up a lot of glucose in the presence of ambient oxygen but convert pyruvate into lactate due to impaired mitochondrial function led to the misconception that cancer cells rely on glycolysis as their major source of energy. Most recent ^{13}C-based metabolomic studies, including in cancer patients, indicate that cancer cells may also fully oxidize glucose. In addition to glucose-derived pyruvate, lactate, fatty acids and amino acids supply substrates to the TCA cycle to sustain mitochondrial metabolism. Here, we discuss how the metabolic flexibility afforded by these multiple mitochondrial inputs allows cancer cells to adapt according to the availability of the different fuels and the microenvironmental conditions such as hypoxia and acidosis. In particular, we focused on the role of the TCA cycle in interconnecting numerous metabolic routes in order to highlight metabolic vulnerabilities that represent attractive targets for a new generation of anticancer drugs.

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Contents

1. Introduction

The Warburg paradigm as the prototypical model of cancer metabolism (*i.e.* preferred aerobic glycolysis and dysfunctional mitochondria in tumor cells) has been the subject of profound reappraisal in the last decade. Cancer cells can indeed use a variety of fuels distinct from glucose to support proliferation and/or survival [1]. Also, accumulating evidence now suggests that mitochondrial metabolism is required for tumorigenesis [2]. Recent studies actually showed that in various tumor types, treatment-resistant cancer cells [3], metastatic/circulating cancer cells [2,4], cancer stem cells (CSC) and tumor-initiating cells (TIC) [5,6] rely on mitochondrial respiration. Moreover, key metabolic enzymes and pathways associated with the mitochondrial metabolism were documented to support tumor progression driven by major oncogenes [7–9].

Heterogeneity in the metabolic preferences was also reported within a given tumor. The mitochondrial oxidation of multiple nutrients may actually co-exist with an enhanced glycolytic pathway (*i.e.* with lactate as end-product) [10]. A role of the tumor microenvironment in the

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oxidative fate of various nutrients was further elicited using *in vivo* isotope tracking [11] and this metabolic heterogeneity in primary tumor was shown to be associated with distinct sites of metastatic spreading [8]. All the above studies have to some extent paved the way of how, by targeting metabolic pathways to which cancer cells may be addicted, eradication of the most life-threatening tumor cells may be envisioned.

1.1. Metabolomic studies in cancer patients shake the Warburg dogma

Stable isotope tracers (*e.g.* [13]C) can be used to investigate cellular metabolism, in particular to track the fate of biosynthetic fuels through the analysis of isotope enrichment downstream of labeled nutrients [12]. This approach has been increasingly used in various mouse models in the last decade [13,14] and more recently in cancer patients. The first human cancers investigated with these tracers are glioma, brain metastases and non-small cell lung cancer (NSCLC) [15,16]; classically, tracers are bolus-administered or infused during surgical resection, and tumor samples are subsequently subjected to ^{13}C NMR spectroscopy. The first observations resulting from these metabolomic studies in cancer patients were that (i) glucose is metabolized not only through glycolysis (*i.e.* up to lactate production) but also through the mitochondrial TCA cycle and (ii) a significant fraction of the acetyl-CoA used in the TCA cycle is not derived from blood-borne glucose [15,16].

More recently, Hensley and colleagues added a layer of sophistication to these studies [10]. They actually assessed tumors in untreated NSCLC patients prior to surgery, using [^{18}F]fluoro-2-deoxy-glucose (FDG) PET imaging and multiparametric MRI including dynamic contrast-enhanced MRI (DCE-MRI) to assess perfusion. Here again, besides the expected enhanced glycolytic flux, they found evidence for oxidation of a large variety of other nutrients. These data further suggest that metabolic heterogeneity between and within tumors can be predicted by assessing tissue perfusion preoperatively. These authors actually proposed that substrates other than glucose would preferentially contribute to the TCA cycle in well-perfused tumor areas whereas glucose oxidation would occur in less perfused tumor regions (where it remains available by virtue of its high concentration and capacity to diffuse). Although there are obvious limitations in the use of DCE-MRI to compare the perfusion in tumors from different patients and in different regions from a same tumor, these results suggest that the local microenvironment may override apparent oncogene-driven metabolic preferences identified in *in vitro* studies. Altogether, the above studies shake the dogma according to which a FDG-PET-positive signal is necessarily correlated with the Warburg effect or so-called aerobic glycolysis. Similar conclusions were recently reached by comparing the fate of isotope-labeled glucose or glutamine in cultured cells *vs.* mouse lung tumors [11]. Deletion of enzymes involved in glucose oxidation (*i.e.* pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC)) actually prevented tumor growth while having no effect on *in vitro* cell growth (see below). Also, while glutaminase (GLS) deletion had no effect on tumor burden *in vivo*, tumor-derived cell lines were highly glutaminolytic and sensitive to GLS inhibitors [11].

The application of metabolomics to cancer patients is still in its infancy and some issues still need to be addressed, such as the presence of stromal cells in the collected tumor fragments and their exact contribution to the measured metabolite enrichment. Other issues would deserve more attention. For instance, the ligation of feeding arteries prior to resection could confuse the interpretation of the preferential use of ^{13}C-labeled nutrients in tumors of cancer patients. Also, the organ of cancer origin could account for major differences because metabolism is optimized in each tissue to match physiological functions and energy/fuel requirements. Pre-existing metabolic peculiarities of a given tissue will thus combine with oncogenic rewiring of the metabolism to support bioenergetic and biosynthetic needs of a given cancer. This complicates the task to associate metabolic preferences with a cancer type or an oncogene. For instance, MYC-driven liver and triple-negative breast cancers are more glutamine-dependent than MET-driven liver

and MYC-driven lung tumors [14,17,18]. Besides these caveats, *in vivo* metabolic and metabolomic studies point towards mitochondria as an unavoidable target in the design of further therapeutic strategies aiming to interfere with the metabolism of glucose, lactate, fatty acids and glutamine (Fig. 1).

1.2. Mitochondria represent a necessary hub in the cancer cell bioenergetics network

The identification of mutations in enzymes of the TCA cycle including succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH) [19,20], has emphasized how alterations in the mitochondrial component of cancer cell metabolism may dramatically alter cell bioenergetics and offer new therapeutic avenues. In cancer cells that do not exhibit mutations in the TCA cycle enzymes, mitochondria operate as a central hub of both catabolic and anabolic metabolism in cancer cells. Here, we outline the critical biosynthetic functions served by mitochondria within tumors with a focus on the peculiarities that may render some metabolic pathways druggable, *i.e.* differentially regulated (*vs.* healthy tissues). The goal of this review is however not to discuss about mitochondrial electron transfer chain (ETC) as a potential therapeutic target and about drugs that in part *via* inhibition of ETC complexes (*e.g.* metformin or phenformin) may perturb mitochondrial oxidative phosphorylation (OXPHOS). Instead, we chose to put into perspective recent insights in the understanding of the TCA cycle fueled by pyruvate, lactate, glutamine and fatty acids (Fig. 1), and the effects (and limitations) of drugs targeting these pathways.

2. Pyruvate reductive and oxidative metabolism

The last step of glycolysis is the production of pyruvate by pyruvate kinase (PK). Pyruvate can either be reduced in the cytosol or transported into mitochondria to be oxidized. Pyruvate reduction into lactate through LDHA generates NAD^+ that is required to restore the NAD^+ pool to maintain a high glycolytic rate (Fig. 2). Of note, LDH
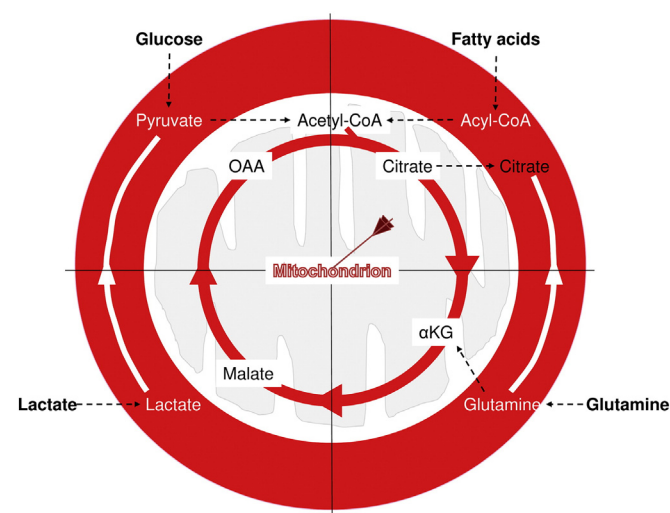
Fig. 1. Mitochondrial metabolism as a therapeutic target. Mitochondria represent a hub for a variety of biosynthetic and bioenergetic pathways fueled by glucose, pyruvate, fatty acids and glutamine. Although part of the glucose is converted into lactate to support a high glycolytic rate, there is now enough evidence to claim that the activity of the TCA cycle, coupled or not to oxidative phosphorylation, represents an obligatory support to tumor cell growth. Addictions to TCA-related metabolic pathways driven by specific oncogenes or the selective pressure of the microenvironment (*e.g.* acidosis, moderate hypoxia, nutrient availability) therefore represent a source of potential targets for a new category of anticancer drugs.
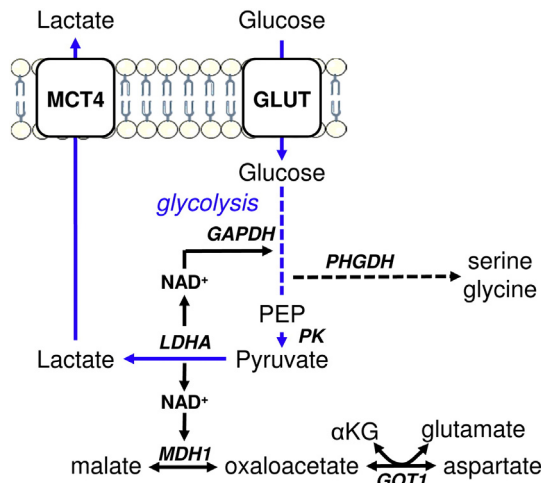
Fig. 2. Non-oxidative glucose metabolism. Under hypoxia or in response to oncogenes (the so-called Warburg effect), glucose is converted into pyruvate and further reduced into lactate by lactate dehydrogenase (LDHA). Associated production of NAD^+ contributes to maintain a high glycolytic rate but also to generate oxaloacetate (via MDH1 or malate dehydrogenase 1) and consecutively aspartate (via glutamic oxaloacetate transaminase or GOT1). GLUT and MCT transporters (in particular MCT4) regulate the influx of glucose and the efflux of lactate, respectively.

enzymes are homo- or hetero-tetramers composed of M and H protein subunits that are encoded by LDHA and LDHB genes, respectively [21]. LDHA proteins actually refer to LDH enzymes with a high M-subunit content that is more favorable to the reduction of pyruvate into lactate (than the opposite) [21]. To maintain a high glycolytic flux, lactate has also to be transported out of the cells according to its concentration gradient. MCT4, a low-affinity high-selectivity transporter for lactate is particularly adapted to exert this function [21].

Based on several clinical insights including a very high degree of FDG uptake detected by PET imaging [22] combined with elevated MCT4 expression [23], triple-negative breast cancer may be considered as addicted to the glucose-to-lactate route. The serine synthesis pathway, a side branch of glycolysis, is particularly activated in this breast cancer subtype with increased expression of 3-phosphoglycerate dehydrogenase (PHGDH) [24,25]. Another glycolytic connection with amino acid production is through the pyruvate-derived NAD^+ which may activate cytosolic malate dehydrogenase (MDH1) to provide oxaloacetate (OAA) from malate and drive aspartate synthesis via glutamic-oxaloacetic transaminase 1 (GOT1) (also named aspartate aminotransferase 1, AST1). This pathway participates into the rescue of cell proliferation when ETC is inhibited [26,27] (Fig. 2).

In the oxidative route, pyruvate is first transported into mitochondria via the mitochondrial pyruvate carrier (MPC), which is composed of the MPC1 and MPC2 subunits located in the inner mitochondrial membrane (Fig. 3). In colon cancer cells, a decreased expression and activity of MPC was found to be critical for tumor xenograft growth in vivo [28]. Other studies however showed that MPC inhibitors, such as UK-5099, can be used in combination with inhibitors of glutamine and fatty acid metabolism, to significantly impair oxidative metabolism in tumor cells as well as proliferation [29,30]. The absence of MPC does not mean that cytosolic pyruvate cannot reach the mitochondrial matrix. Pyruvate is actually interchangeable to alanine or malate by single reactions, catalyzed by ALT or malic enzyme (ME), respectively, in both the cytosol and mitochondria (Fig. 3). Two recent studies actually reported that pyruvate-alanine cycling may bypass MPC in hepatic cells [31,32]. Although this has not yet been formally demonstrated in tumor models, these pathways could contribute to MPC-independent pyruvate fueling of the TCA cycle and thus represent adaptive responses to MPC inhibitors.
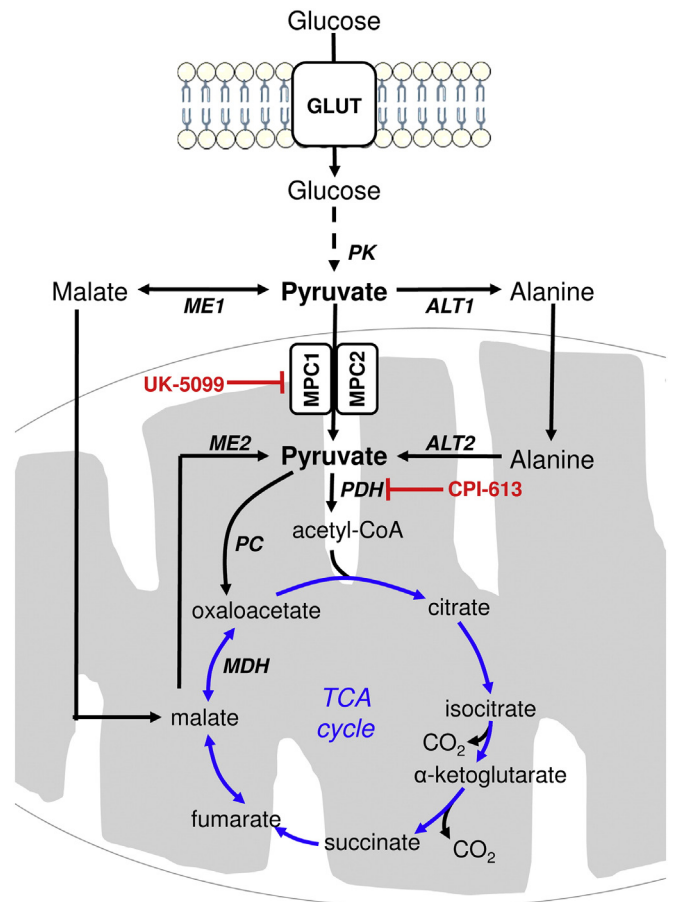
Fig. 3. Glucose-derived pyruvate oxidative metabolism. Glucose-derived pyruvate may enter into the mitochondria through MPC (mitochondrial pyruvate carriers 1 and 2) to be further oxidized. Pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) give rise to acetyl-CoA and oxaloacetate, respectively. Besides this route, pyruvate can also enter mitochondria after interconversion into malate and alanine (via malic enzymes ME1 and ME2, and alanine aminotransferase ALT1 and ALT2, respectively). Drugs under clinical development or used as preclinical tools are indicated in red.

After reaching the mitochondrial matrix, pyruvate can be oxidized by the pyruvate dehydrogenase (PDH) complex to form acetyl-CoA which is then condensed with OAA to form citrate (Fig. 3). Pyruvate can also be converted by pyruvate carboxylase (PC) to form OAA and thereby offer an alternative anaplerotic reaction to glutamine-fueled TCA cycle (see below). This mitochondrial metabolism of pyruvate through PDH and PC was found to be essential in mice for the formation of KRAS-driven lung tumors [11]. PC was also recently reported as a major anaplerotic route in breast cancer-derived lung metastases (vs. primary breast tumor) [33]. The above data actually confirmed previous work in cancer patients. Sellers and colleagues had indeed documented that in NSCLC patients the major source of anaplerosis to replenish TCA cycle precursor intermediates was glucose (through pyruvate) and not glutamine [9].

The anti-cancer action of the off-patent small molecule dichloroacetate (DCA) has introduced some confusion in the role of pyruvate oxidation in supporting cancer cell proliferation. DCA was indeed initially reported as a PDK inhibitor (and thus a PDH activator) promoting a shift from glycolysis to respiration that was associated with pro-apoptotic effects [34]. Although this was confirmed by several independent laboratories working on various cancer cell lines [35], the intimate link between glucose and fatty acid oxidation (see below) could actually also be involved in DCA effects because of mitochondrial FA oxidation being altered in response to the shift in glucose metabolism (i.e. instead of a direct growth inhibitory effect resulting from a reduced glycolytic

flux). It should also be noted that in some studies, DCA was reported to promote cancer progression [36,37] and conversely, the PDH inhibitor CPI-613 was recently reported to exert anticancer effects [38]. More generally, the pre-existing *in vitro* glycolytic drift of many cell lines has also led to overestimate the therapeutic potential of DCA. This issue is actually a major misleading fact that has contributed to make glycolysis the champion metabolic path in cancer cells. The plastic life of numerous cancer cell lines with full access to glucose and buffered medium has indeed largely contributed to associate the tumor metabolic phenotype as exquisitely glycolytic and limitedly dependent on oxidative phosphorylation. We and others have shown that when tumor microenvironment conditions (*e.g.* acidosis, low glucose) are recapitulated *in vitro*, aerobic glycolysis may even become a minor metabolic path while TCA cycle and oxidative phosphorylation largely support cell proliferation [39–41]. As emphasized above, this observation is supported by recent metabolomic studies in cancer patients that provide evidence for full oxidation of glucose into CO_2 and the contribution of various nutrients to acetyl-CoA in tumor metabolism [15,16].

3. Lactate as an alternate source of pyruvate

Oxidative tumor cells can use lactate instead of (or in addition to) glucose as an energy source. This requires the cellular uptake of lactate through monocarboxylate transporters (MCTs) and the conversion of lactate into pyruvate by LDHB (Fig. 4). At the difference of LDHA proteins (see above), LDHB proteins refer to LDH enzymes with a high H-subunit content that is more favorable to the oxidation of lactate into
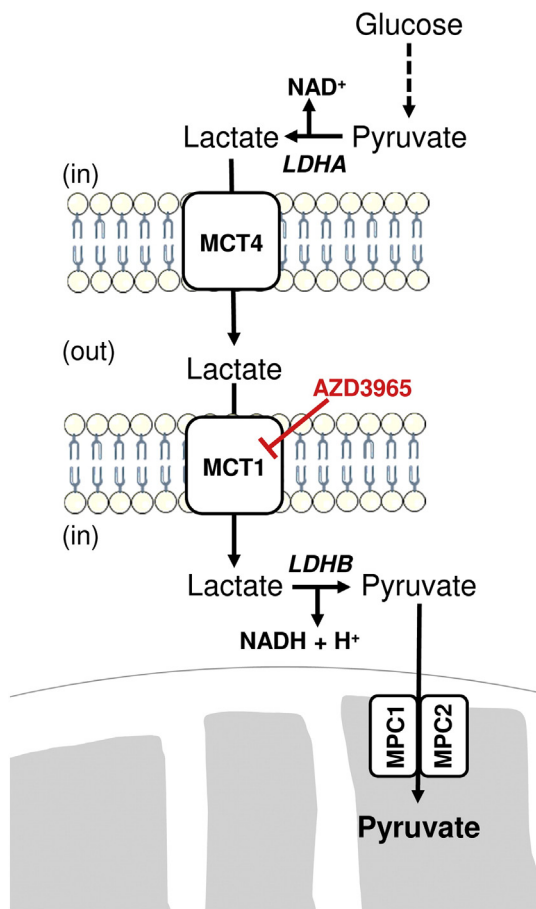
pyruvate. In tumors, hypoxic (glycolytic) tumor and/or stroma cells consume glucose and release lactate through MCT4 (Fig. 4). Lactate can be taken up by tumor cells (particularly in the perivascular compartment) but also by tumor-associated cells such as endothelial cells and fibroblasts *via* MCT1 [42–45]. In this better oxygenated environment, lactate can be oxidized into pyruvate and further metabolized aerobically (Fig. 4). This metabolic symbiosis offers new perspectives to therapeutically target the hypoxic tumor microenvironment and to tackle tumor angiogenesis [21,42,46,47]. Recently, three studies have highlighted the role of lactate-driven metabolic symbiosis in tumor resistance to antiangiogenic treatments [48–50]. These authors showed that adaptive response to antiangiogenic drugs could be blocked by disrupting the glycolytic flux through administration of the glycolysis inhibitor 3-PO or *via* MCT4 knock-down [48,50] as well as by inhibiting mTOR that supports the MCT1-dependent component of the symbiotic compartmentalization [48,49]. Another study also documented that estrogen-positive breast cancer cells could switch to lactate as a primary source of energy to survive glucose deprivation and that this metabolic pathway regulated by the estrogen-related receptor alpha (ERRα) conferred resistance to PI3K/mTOR inhibitors [51]. Interestingly, although non-hormonal triple-negative breast cancer is described as highly glycolytic (see above), an integrated genomic screen identified LDHB as essential for the progression of this type of cancer [52]. In the same study, MCT1 was co-expressed in LDHB-high tumors suggesting that these tumors may re-capture and metabolize lactate that they have themselves generated in more favorable glucose-replete conditions. Altogether these observations strengthen the idea that the blockade of lactate import *via* MCT1 represents an attractive therapeutic strategy. AZD3965, a chemical inhibitor of MCT1 and MCT2, is currently being evaluated in the clinics (Fig. 4); whether such compound is compatible with the critical role of lactate in the brain [21,53] has however still to be determined.

4. Fatty acid metabolism

Fatty acids constitute a prominent source of anabolic substrates and reducing equivalents for tumor cells. After uptake *via* CD36/FAT transporter and conversion into acyl-CoA through the long-chain-fatty-acid-CoA ligase 1 (ACSL1), they are transported into mitochondria, *via* the carnitine palmitoyl transferase-1 (CPT1), where they undergo beta-oxidation generating acetyl-CoA (that feeds into the TCA cycle) (Fig. 5) but also reducing equivalents (NADH + H^+ and $FADH_2$) for oxidative phosphorylation. A recent study identified fatty acid oxidation (FAO) as being upregulated in MYC-overexpressing triple-negative breast cancer [7]; inhibition of FAO by etomoxir, a CPT1 blocker, displayed significant anticancer effects *in vivo*. This agent showed antitumor effects in other tumor models such as glioblastoma and acute myeloid leukemia [54,55]. Clinical trials using etomoxir however revealed severe hepatotoxicity and had to be stopped [56]. Other drugs blocking fatty acid uptake and/or oxidation are currently under development or already used in the clinics to treat cardiac diseases including heart failure and diabetic cardiomyopathy. The inter-regulation between FA and glucose metabolism, a process referred to as the Randle cycle allows to partly understand how reducing FA metabolism may be beneficial in these circumstances. Indeed, according to the Randle's paradigm, while increasing glucose oxidation inhibits FAO in the heart, increasing FAO decreases glucose oxidation and favors glycolysis. The latter being detrimental for heart metabolism, pharmacological inhibition of FAO was proven to be beneficial to treat cardiac diseases. Repurposing of these cardiac drugs could thus speed up their clinical evaluation in cancer patients. Current available drugs are inhibitors of malonyl-CoA decarboxylase (*e.g.* CBM-301106), CPT-1 (*e.g.* perhexiline, ST1326) and 3-ketoacyl coenzyme A thiolase also named acetyl-CoA C-acyltransferase (ACAA2) (*e.g.* trimetazidine) [57] (Fig. 5).

Although FAO represents an important route to generate acetyl-CoA as well as NADH and $FADH_2$ molecules that may support cancer cell
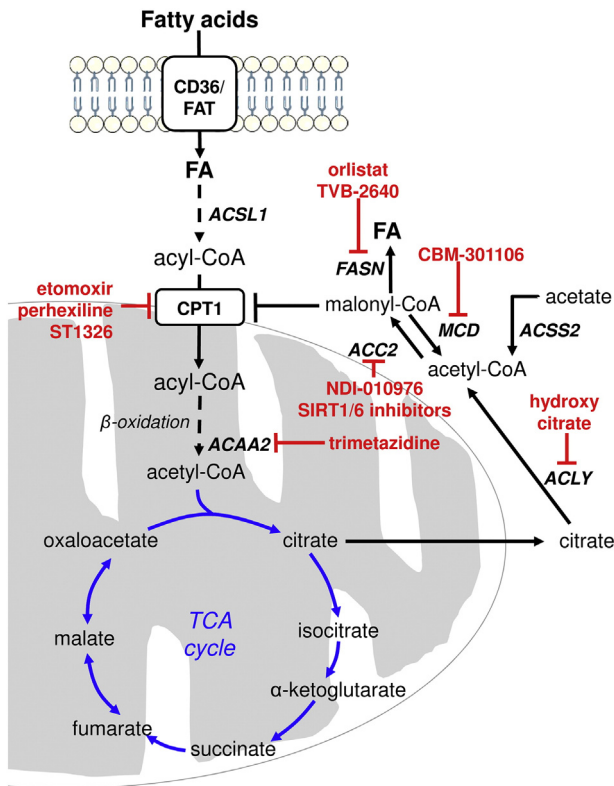

Fig. 4. Lactate oxidative metabolism. Lactate released by the most glycolytic cells in tumors (through monocarboxylate transporter MCT4) can be captured by cancer cells (most often through MCT1) to be oxidized into pyruvate by lactate dehydrogenase B (LDHB). Pyruvate then follows the paths detailed in Fig. 2. Drugs under clinical development or used as preclinical tools are indicated in red.

Fig. 5. Fatty acid metabolism. Fatty acid (FA) oxidation requires the uptake of FA through plasma membrane transporters CD36/FAT and the transport into mitochondria *via* carnitine palmitoyl transferase-1 (CPT1) after transformation into acyl-CoA *via* long-chain-fatty-acid-CoA ligase-1 (ACSL1). Acyl-CoA is then progressively oxidized into acetyl-CoA that fuels the TCA cycle. FA synthesis requires citrate conversion into acetyl-CoA *via* ATP citrate lyase (ACLY) and further polymerization *via* acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) enzymes. Drugs under clinical development or used as preclinical tools are indicated in red.

survival and proliferation, *de novo* synthesis of fatty acids (lipogenesis) is also needed to produce new phospholipid bilayers. Several steps are required to convert citrate to bioactive fatty acids and some enzymes involved in this pathway, including ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN) have been associated with tumor development and progression *in vitro* and *in vivo* [58] (Fig. 5). Drugs targeting the above three enzymes (see Fig. 5) are currently under evaluation [59–61]. Interestingly, the FA synthesis inhibitor orlistat was recently documented to inhibit tumor regrowth and metastases after treatment with the antiangiogenic compound sunitinib [62].

For FA synthesis, citrate produced from pyruvate-derived acetyl-CoA needs to be transported into the cytosol *via* the citrate transporter protein (CTP or SLC25A1). Citrate can also be generated upon reductive carboxylation of glutamine (see below), thereby offering an alternate source of fatty acids in particular when glucose is not available or in response to hypoxia [63,64] or acidosis [39,65]. Interestingly, under acidosis, FA oxidation and synthesis were shown to co-exist in cancer cells through the histone deacetylation-mediated downregulation of acetyl-CoA carboxylase ACC2, a mitochondrion-anchored enzyme that normally prevents the degradation of neo-synthesized FA [39]. In healthy tissues, fatty acid synthesis (FAS) and oxidation (FAO) are indeed two mutually exclusive pathways because of the regulatory effects of malonyl-CoA, which is involved in FA elongation but also negatively regulates FAO by inhibiting CPT1 [66]. Drugs targeting sirtuins 1 and 6 by blocking histone deacetylation were shown to prevent ACC2 downregulation and to lead to tumor growth inhibitory effects [39]. Of note, in the same study related to the metabolic shift occurring under acidosis, FA-derived acetyl-CoA was shown to fuel the TCA cycle and to support tumor cell respiration but also to contribute to non-enzymatic

acetylation of mitochondrial ETC complex I, thereby restraining ROS production. Finally, it should be emphasized that upon nutrient deprivation, acetyl-CoA may also be generated from one of the simplest carboxylic acid, namely acetate, *via* the action of the cytosolic enzyme acetyl-CoA synthase 2 (ACSS2) [67].

5. Mitochondrial metabolism of glutamine and other amino acids

Cancer cells usually rely on glutamine (*i.e.* not on glucose) to replenish the TCA cycle. Anaplerosis is supported by the double deamination of glutamine into alpha-ketoglutarate (αKG) through the consecutive action of glutaminase (GLS) and glutamine dehydrogenase (GDH) (Fig. 6). Interestingly, DeBerardinis and colleagues have documented that glucose deprivation or inhibition of MPC stimulates a pathway in which acetyl-CoA and OAA can be formed from glutamine (Fig. 6) and conversely that import of pyruvate into the mitochondria suppresses GDH and glutamine-dependent acetyl-CoA formation [30]. Malic enzyme ME2 which decarboxylates malate to pyruvate was shown to account for the ability of glutamine to support TCA cycle activity as a sole carbon source (Fig. 6). Such capacity to supply both OAA and acetyl-CoA to maintain TCA cycle activity regardless of glucose or pyruvate availability makes glutamine a unique fuel for anaplerosis; fatty acids can for instance provide acetyl-CoA but not OAA. Although this potential major role in cancer metabolism may be tumor-dependent [11], glutaminase inhibition (*e.g.* by BPTES and its derivative CB-839) and glutamine depletion using L-asparaginase represent promising therapeutic strategies [68,69]. It should also be emphasized that several distinct routes can generate αKG from glutamine. First, cytosolic and mitochondrial glutaminases, GLS1 and GLS2 respectively, can lead to the formation of glutamate, which may in turn be converted into αKG through deamination by GDH but also by transamination *via* alanine and aspartate transaminases ALT and AST (also called glutamic pyruvate transaminase GPT and glutamic oxaloacetate GOT2, respectively); both transaminases also exist as cytosolic and mitochondrial forms (Fig. 6). In some cancer types including NSCLC, branched chain amino acids (BCAA) valine, leucine and isoleucine can also be metabolized by transaminases (BCAT1) in both compartments [70]. More generally, the carbon skeletons of so-called ketogenic and glucogenic amino acids can be transformed into acetyl-CoA and TCA cycle intermediates, respectively (Fig. 6). This explains how autophagy can indirectly fuel the TCA cycle by supplying amino acids (from protein degradation) and thereby contribute to survival during exogenous nutrient deprivation.

Glutamine also represents a source of citrate through non-canonical carboxylation of αKG; this pathway preferentially leads to fatty acid synthesis upon citrate cleavage into acetyl-CoA. This reductive carboxylation represents the major metabolic route for glutamine when hypoxia prevents ETC or in the presence of mutations in the TCA cycle downstream αKGDH [63,64,71]. The duality of enzymes such as GLS and GDH but also IDH, which exist as cytosolic and mitochondrial forms, however allows both routes to occur concomitantly (Fig. 6). In particular, Corbet and colleagues reported that under acidosis (as encountered in most tumors *in vivo*), αKG can be handled in the cytosol to provide a source of acetyl-CoA to support fatty acid synthesis and at the same time to fuel the mitochondrial TCA cycle through the generation of OAA and pyruvate [65].

Finally, when the intracellular glutamine supply exceeds the demand, glutamine can be exchanged for essential amino acids to stimulate mTORC1 and protein synthesis [72]. Together with the generation of glutamate that contributes to nonessential amino acid synthesis *via* transamination, this may position glutamine as a major actor of protein biosynthesis in some cancer types. Importantly, although glutamine addiction is usually associated with anaplerosis, Tardito and colleagues reported that, in glioblastoma, glutamine is dispensable as a TCA fuel [73]. These authors further documented that, in glioblastoma, glutamine is actually synthesized from glutamate through the action of glutamine synthetase and critically contributes to nucleotide synthesis. The
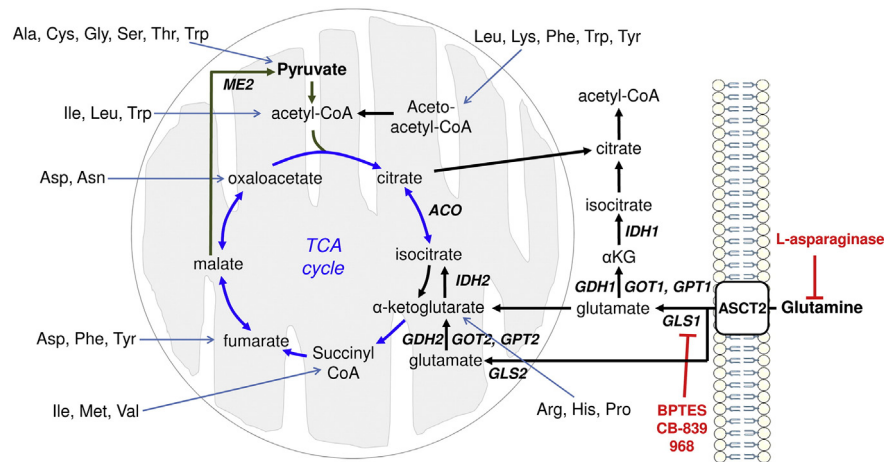
Fig. 6. Glutamine metabolism and amino acid contribution to the TCA cycle. After uptake through a variety of transporters including ASCT2, glutamine can follow a similar fate into the cytosol and the mitochondria. Two consecutive deaminations into glutamate *via* glutaminase (GLS) and then into α-ketoglutarate through one of the three following enzymes: glutamate dehydrogenase GDH, glutamic oxaloacetate transaminase GOT and glutamic pyruvate transaminase GPT, also named ALT; cytosolic and mitochondrial forms of these enzymes exist. In the mitochondria, α-ketoglutarate will be converted into succinyl-CoA and then succinate and will thereby support anaplerosis, or it will be carboxylated into isocitrate to give rise to citrate. The latter pathway called "reductive carboxylation" also occurs in the cytosol and represents a major source of citrate to generate acetyl-CoA and fatty acids under hypoxia, acidosis or when conventional TCA cycle is blocked. Amino acids, when deaminated, yield ketoacids that, directly or *via* additional reactions, feed into the TCA cycle. The catabolism of so-called glucogenic amino acids can produce either pyruvate or some TCA cycle intermediates while the catabolism of ketogenic amino acids produces acetyl-CoA (or acetoacetyl-CoA). Drugs under clinical development or used as preclinical tools are indicated in red.

mechanisms of glutamine dependence were also addressed in breast cancers [74]. Luminal- but not basal-type breast cancer cells were identified by these authors as independent of glutamine uptake. Luminal-specific expression of glutamine synthetase was shown to further re-press glutaminase expression.

6. Mitochondrial metabolism and ROS production

Although the above paragraphs support the hub function of mito-chondria to regulate cancer cell metabolism, these organelles also represent the major locales for the production of reactive oxygen species (ROS) (*i.e.* superoxide anion, hydroxyl radical and hydrogen peroxide), in large part resulting from oxygen-fueled metabolic reactions. One of the best evidence of the contribution of TCA cycle-fueled mitochondrial respiration to ROS arises from the study of uncoupling protein (UCP). UCP2 was indeed reported to limit glucose oxidation (in part by preventing pyruvate entry into mitochondria and by exporting OAA and related C4 compounds from mitochondria [75,76]) and to consecutively reduce ROS production by lowering the redox pressure on mitochondrial ETC. The link between tumor progression, metabolism and ROS is increasingly investigated and several apparently contradictory studies have been reported in the last few years. While some investigators have provided evidence that ROS production supports tumor cell growth [77] and even metastatic spreading [78,79], the field was recently shaken by studies reporting a paradoxical inverse relationship between ROS and cancer progression. Piskounova and colleagues for instance reported that metastatic and circulating cancer cells displayed higher levels of ROS than primary tumor cells but surprisingly that treatment with antioxidant N-acetyl-cysteine increased the occurrence of metastatic burden [80]. Another recent study by Le Gal and colleagues had reached similar conclusions further supporting therapeutic approaches that would exacerbate the pro-oxidant environment of cancer cells (instead of blocking oxidative stress) in order to prevent metastases [81]. These studies are actually making echo to previous large-scale clinical trials that failed to document a positive effect of antioxidant supplementation on cancer incidence, and even provided evidence for a worsening outcome for patients [82].

Again, experimental models and drug specificity (*i.e.* mitochondria-targeting or not) may account for discrepancies between studies reporting detrimental or beneficial effects of antioxidant strategies.

Piskounova and colleagues actually reported that for an efficient metastasis process, a prerequisite for melanoma cells is to undergo metabolic changes to increase NADPH generation in order to gain the capacity to withstand oxidative stress [80]. In the same study, however, implanted metastases but not primary melanomas were sensitive to a variety of antioxidant strategies. In other words, oxidative stress is deleterious for metastasizing cancer cells (*i.e.* on their way to distant organs) making antioxidant drugs inappropriate but when established, metastases become secondary tumors that could benefit of antioxidant treatments. The continuous spreading of cancer cells from primary tumors however makes a global antioxidant approach difficult to set up except maybe strictly after surgical removal of the primary tumor (*i.e.* the source of disseminating cells).

7. Conclusions

Tumor plasticity has never been more true than when applied to metabolism, particularly when referring to the mitochondrial hub where many metabolic pathways converge. The amazingly quite exhaustive knowledge of the interconnected metabolic routes allows today to understand and even anticipate the response of tumors to the pharmacology blockade of a given metabolic enzyme or transporter. This also renders the task of efficiently targeting tumor metabolic preferences very challenging. Development of surrogate markers of tumor bioenergetics appears therefore necessary to determine which pathway(s) to target with the metabolism-targeting drugs soon available. The existence of such biomarker is probably the reason why among the current clinical trials evaluating drugs targeting tumor metabolism, drugs interfering with the rare but easily tractable mutation into the metabolic enzyme IDH were the first to be reported [83,84]. In most situations, however, metabolic enzymes and transporters are not mutated in cancers. To identify predictive biomarkers will thus represent a major challenge if one compares metabolism-targeting drugs with current targeted therapies that are most often designed to target mutated enzymes or receptors that have been identified in the patient tumors. Nevertheless, evidence is increasingly compelling that genetic alterations dictate at least in part metabolic preferences of cancer cells; those could constitute a first basis of cancer patient selection before the development of techniques such as magnetic resonance spectroscopy to non-invasively image metabolites and thereby track metabolism specificities

in patient tumors. It should also be considered that organ-specific oncogene-driven addiction to some metabolic fuels [85] may participate in the safety of administration of drugs targeting metabolic enzymes since this may avoid the disruption of the metabolism in healthy tissues. Ironically, the metabolic plasticity could indeed also be beneficial for normal tissues that could adapt to the blockade of specific metabolic pathway(s) while tumor cells could not. Finally, metabolic studies in human cancer patients have questioned the relevance of *in vitro* work and should certainly stimulate users of these more tractable models to integrate critical *in vivo* parameters such as hypoxia, acidosis and relevant concentrations of major nutrients in their experimental models. This is a must to provide the more-than-a-century old field of cancer metabolism with the knowledge accumulated in the last decades by the oncogene-driven experts and the tumor environmentalists.

Transparency document

The Transparency document associated with this article can be found, in online version.

Acknowledgements

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