Cancer Metastasis Rev
DOI 10.1007/s10555-012-9384-6

NON-THEMATIC REVIEW

Non-glucose metabolism in cancer cells—is it all in the fat?

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Abstract Cancer biologists seem to have overlooked tumor metabolism in their research endeavors over the last 80 years of the last century, only to have "rediscovered Warburg" (Warburg *et al*. 1930; Warburg, Science 123(3191):309–314, 1956) within the first decade of the twenty-first century, as well as to suggest the importance of other, non-glucose-dependent, metabolic pathways such as such as fatty acid *de novo* synthesis and catabolism (β-oxidation) (Mashima *et al*., Br J Cancer 100:1369–1372, 2009) and glutamine catabolism (glutaminolysis) (DeBerardinis *et al*., Proc Nat Acad Sci 104(49):19345–19350, 2007). These non-glucose metabolic pathways seem to be just as important as the Warburg effect, if not potentially more so in human cancer. The purpose of this review is to highlight the importance of fatty acid metabolism in cancer cells and, where necessary, identify gaps in current knowledge and postulate hypothesis based upon findings in the cellular physiology of metabolic diseases and normal cells.

Keywords Tumor metabolism · β-oxidation · Aerobic respiration · Energy metabolism · Tricarboxylic acid

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Published online: 17 June 2012

1 Overview of tumor metabolism

The reprogramming of energy metabolism is one of the "emerging hallmarks" of cancer recently defined by Hannahan and Weinberg [1]. Otto Warburg was the first to demonstrate that anaerobic metabolism of glucose occurred in the presence of oxygen (aerobic glycolysis) in cancer cells, but not in normal untransformed cells [2, 3]. Importantly, he also showed that aerobic respiration, what we know today as mitochondrial oxidative phosphorylation (OXPHOS), occurred concurrently with aerobic glycolysis. Therefore, it is not correct to suggest that aerobic glycolysis is the exclusive ATP-generating system in cancer cells. In reality, *in vivo* tumors probably rely on both aerobic glycolysis and OXPHOS for their energy, the proportions of which are probably dependent on both the genetic background of the cancer cell (*e.g.*, c-MYC oncogene expression and *TP*53 mutation), as well as its microenvironment [4], particularly intratumoral hypoxia, which induces hypoxia-inducible factor (HIF)-1α expression, an *a priori* transcription factor that in turn upregulates glycolytic enzyme gene expression [5].

2 Non-glucose substrates in tumor metabolism

Mitochondria are not only the seat for the electron transport chain but they are also the site for the tricarboxylic acid (TCA; Kreb's) cycle. We will describe in this review article how non-glucose substrates and their cognate pathways that feed into the TCA cycle may be important oncogenic metabolic pathways in their own right and are possibly on a par to aerobic glycolysis in certain contexts as regards their importance within the cancer cell metabolome.

The TCA cycle is the cancer cell's principal "biochemical hub" where different carbon sources are substrates for both

OXPHOS and cellular biosynthesis such as nucleotide production for DNA synthesis/repair and cellular membrane synthesis [6]. This probably endows cancer cells with a metabolic plasticity that may be essential to ensure survival within the nutrient-depleted microenvironment of the growing tumor. An increasing weight of both *in vitro* and *in vivo* evidence suggests that in particular tumors, such as glioblastoma [7] and prostate cancer [8, 9], cancer cells might be more reliant on these non-glucose carbon sources, even in glucose-replete conditions. This would be consistent with the fact that cancer cells, unlike normal cells, demonstrate an increase in the expression of cytoplasmic and mitochondrial enzymes involved in *de novo* fatty acid synthesis, or glutaminolysis, which seems to be primarily used for cellular biosynthetic processes. However, the carbon source for this (glucose *versus* fatty acids *versus* glutamine) may be dependent on cellular microenvironmental conditions, as well as the background metabolic programming of the cancer cell. In untransformed cells, mitochondrial fatty acid β-oxidation generates NADH, which provides essential reducing equivalents to drive OXPHOS. Importantly, NADH concomitantly suppresses mitochondrial pyruvate dehydrogenase (PDH), the enzyme of greatest control strength over the entry of pyruvate into the TCA cycle *via* acetyl-CoA. Fatty acid β-oxidation-derived acetyl-CoA also suppresses PDH activity by increasing the acetyl-CoA/CoA ratio, and this, together with a high mitochondrial NADH/NAD ratio, attenuates the oxidation of pyruvate, thus inhibiting glucose oxidative metabolism. Consequently, cytoplasmic pyruvate is diverted to lactate and induces the characteristic metabolic signature of cancer cells originally described by Warburg. Although we now have a greater understanding of the regulation of the Warburg effect at a molecular level, the mitochondrial metabolic reprogramming which accompanies β-oxidation [10] might in fact have a Warburg-*ian* effect which augments the conversion of glucose into lactate [2, 1].

3 Fatty acid metabolism

In normal untransformed cells, the balance between FA synthesis and β-oxidation is dependent upon nutritional state and tissue mitochondrial metabolism. In a nutrient-sufficient extracellular milieu, the majority of FA substrates are derived from exogenous fatty acids and are actively transported into the cell [11]. In contrast, because of nutritional deprivation due to limited vascular supply, cancer cells rely on *de novo* FA synthesis for generating these substrates for cellular biosynthesis and β-oxidation. The synthesis of FAs starts with the mitochondrial export of citrate which is produced by glutaminolysis. Mitochondrial glutaminase converts glutamine to glutamate which is then transaminated to mitochondrial α-ketoglutarate (mitochondrial 2-oxoglutarate) to produce citrate *via* the TCA cycle. A proportion of this citrate is exported into the cytoplasm by the SLC25A1 mitochondrial citrate uniporter. Subsequently, cytoplasmic citrate is metabolized into acetyl-CoA and oxaloacetate by ATP-citrate lyase (ACLY), the expression of which is increased in cancer cells compared to normal cells and is possibly an important determinant for tumor growth [12].

4 *De novo* fatty acid synthesis and cytoplasmic metabolism of saturated long-chain fatty acids

Once acetyl-CoA is in the cytoplasm, it is metabolized by acetyl-CoA carboxylase (ACC) to yield malonyl-CoA, the first committed precursor of saturated long-chain fatty acid (LCFA) biosynthesis. Malonyl-CoA is subsequently metabolized to LCFAs, such as palmitic acid, which is the most abundant LCFA in cancer cells, by the NADPH-dependent enzyme—fatty acid synthase (FASN). LCFAs can be subsequently desaturated to monounsaturated fatty acids (MUFAs) by stearoyl-CoA desaturase (SCD)-1 [13, 14, 15]. MUFAs are important for tumorigenesis because they are important constituents of the phospholipid cell membrane and are thought to affect its biomechanical integrity [16]. Inhibition of MUFA synthesis by SCD-1 inhibitors, such as CVT-11127 [17], has been proposed as a strategy for reducing tumor growth by attenuating cell proliferation at the G_1/S cell cycle phase and initiating apoptosis [18], although concomitant inhibition of ACC in lung cancer cells does not augment the suppression of cell proliferation by SCD-1 inhibition alone [18].

In cancer cells, LCFAs which are not metabolized to MUFAs are destined for two fates—cytoplasmic storage as glycerolipids or mitochondrial import for β-oxidation. The former involves the transient storage of palmitic acid as mainly cytoplasmic glycerolipid monoacylglycerol (MAG). MAG seems to be part of an important cellular homeostatic mechanism that prevents lipotoxicity within cancer cells with high levels of FA synthesis since palmitic acid can have both mitochondrial and endoplasmic reticulum-mediated pro-apoptotic effects which are probably cell type dependent. Moreover, given that certain high-grade, but not low-grade, tumors express high levels of monoacylglycerol lipase (MAGL) which metabolizes MAG back to palmitic acid [19], increased expression of MAGL might be a biochemical adaptation in high-grade tumors to facilitate high levels of β-oxidation which would consequently reduce the amount of free cytoplasmic palmitic acid, thus preventing the deleterious effects of palmitic acid to cancer cell survival.

5 Molecular regulation of fatty acid metabolism

The two major non-mutually exclusive upstream regulators of fatty acid metabolism that interconnect FA metabolism to tumor growth are AKT [20–22] and mTORC1 [20, 23–25]. Activated AKT signaling increases overall levels of FA synthesis by upregulating expression of the master lipid transcription factor, sterol regulatory element-binding protein (SREBP), which in turn increases the expression of enzymes which are required at different stages of FA synthesis, such as FASN [21] and SCD-1 [25] (Fig. 1). Furthermore, there are three lines of evidence to suggest that activated AKT increases LCFA synthesis at the expense of β-oxidation: (1) inverse relationship between FASN expression and PTEN status (the endogenous tumor suppressor for PI3K/AKT) in epithelial cancer cells [26], (2) MUFA upregulates microRNA-21 expression which attenuates PTEN expression, thus augmenting AKT-dependent FASN expression [27], and (3) AKT downregulates the expression of the principal mitochondrial membrane LCFA importer carnitine palmitoyltransferase (CPT)-I, thereby reducing β-oxidation [28]. Therefore, it could be hypothesized that in $PTEN^{-/-}$ cells, where AKT is constitutively activated, and would therefore be expected to demonstrate greater rates of cell proliferation compared to their isogenic $PTEN^{+/+}$ counterparts, LCFAs could be utilized primarily for cellular membrane biosynthesis, rather than for β-oxidation-dependent anapleurosis and ATP generation. Interestingly, MUFA which are not utilized for membrane biosynthesis are possibly involved in a positive feedback loop that replenishes levels of LCFA in a miR-21-dependent manner which increases the levels of activated AKT (Fig. 1), thus increasing SREBP-1-dependent transactivation of multiple FA metabolism genes. Additionally, apart from the independent and cooperative effects of SREBP-1c and HIF-1α [26], FASN expression can also be regulated by p53, p53 homologs (*e.g.*, p63) [29] and SPOT14 [30], as well as proteins which contribute to the stabilization of FASN, such as isopeptidase USP2a [31]. In contrast, in human prostate cancer cells, high levels of FASN protein expression is a direct consequence of increased FASN gene copy number gains [32], although changes in FASN or any other FA metabolism gene copy number have not been reported to date in any other human cancer.

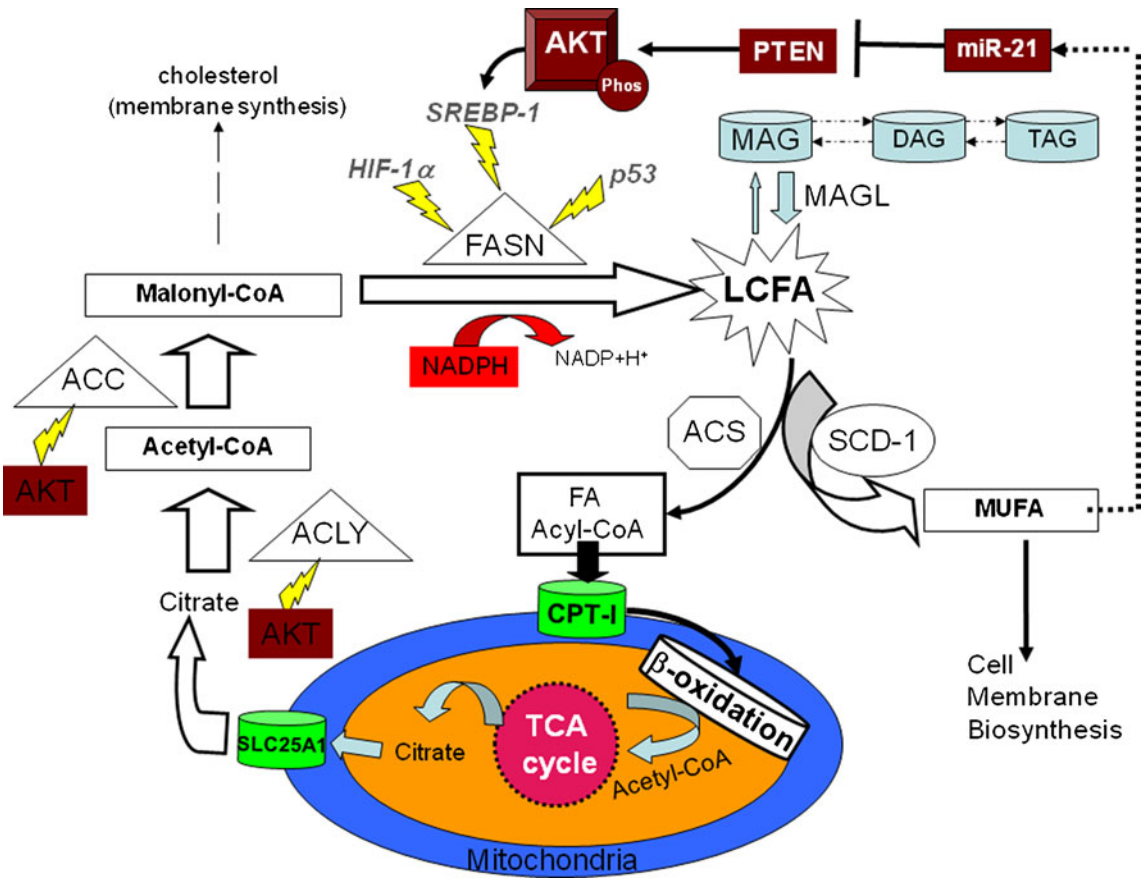

Fig. 1 The molecular regulation of *de novo* fatty acid synthesis and the biochemical fate of saturated long-chain fatty acids (*ACLY* citrate lyase, *ACC* acetyl-CoA carboxylase, *FAS* fatty acid synthase, *SREBP-1* sterol regulatory element binding protein-1, *LCFA* long-chain fatty acids, *ACS* acyl-CoA synthase, *SCD-1* stearoyl-CoA desaturase, *MAGL* monoacylglycerol lipase, *CPT-I* carnitine palmitoyltransferase-I)

As regards transcriptional regulation, the predominant SREBP isoform in cancer cells is SREBP-1c [24, 26, 33], which seems to be more dependent on mTORC1 signaling rather than direct upstream AKT signaling. Rapamycin treatment of *in vitro* breast cancer cells has demonstrated that mTORC1 can also independently transactivate the SCD-1 promoter [25], thereby increasing MUFA synthesis. Given that HIF-1α can also independently upregulate SREBP-1 and FASN expression [26], and that mTORC1 is also necessary for HIF-1α translation, rapamycin and its analogs (rapalogs) could therefore possibly attenuate *de novo* fatty acid synthesis at multiple molecular regulatory levels during tumor growth and its hypoxia sequela, through attenuation of HIF-1α translation [25], as well as reducing the induction of FASN expression caused by the epigenetic modifying by-products of the Pasteur effect in cancer cells which causes hypoxia-induced extracellular acidosis [34]. On a cautionary note, given that rapalogs can trigger the feedback activation of AKT, abrogation of FA metabolism might not be therapeutically achievable with rapalogs alone.

6 The putative role of fatty acid synthesis in oncogenesis and premalignant cell transformation

Currently, very little is understood about the role of FA metabolism in tumorigenesis. However, it is possible that the production of cytoplasmic NADPH might be an important homeostatic regulator of both FASN expression and activity [35]. NADPH is produced as a by-product of the isocitrate dehydrogenase (IDH)-1 dependent metabolism of isocitrate to cytoplasmic α-ketoglutarate (cytoαKG). CytoαKG is an important co-substrate for prolyl hydroxylase (PHD), a dioxygenase that is upregulated by HIF-1α in a positive feedback loop and which mediates the proteosomal degradation of HIF-1α [36], thus preventing its stabilization in normoxia. Metabolically, this in turn prevents the transactivation of HIF-1α-mediated metabolism genes, involved in fatty acid synthesis (*e.g.*, FASN), as well as maintaining pyruvate oxidation in normoxia through the loss of induction of HIF-1α-dependent lactate dehydrogenase (LDH) and pyruvate dehydrogenase kinase (PDK)-1 transcription (Fig. 2).

Approximately 70 % of low-grade gliomas and secondary glioblastomas [37] and a significant minority of acute myeloid leukemia [38] have a mutation in either of two known closely related IDH genes—IDH1 (R132) or IDH2 (R142 or R170). The oncogenic effects of mutated IDH genes are dominant gain-of-function effects which appear phenotypically to be similar, despite their different subcellular localizations, such that IDH1 resides within the cytoplasm and IDH2 is located within mitochondria. The mechanistic effects of IDH1 R132 are the best described and involve two synergistic mechanisms. First, IDH1 R132 mediated increase of the metabolism of cytoαKG to D-2-hydroxyglutarate (D-2-HG), a putative onco-metabolite [37, 39, 40] (Fig. 3a). Second, a reduction in the overall synthesis of αKG by reducing the metabolism of isocitrate to cytoαKG, thus depriving PHDs of an essential co-factor, thereby triggering HIF-1α stabilization and its downstream oncogenic effects (Fig. 3b). However, mutated IDH1 could also be hypothesized to have suppressive effects on tumor growth by reducing NADPH levels [39, 41], thus attenuating NADPH-dependent FASN activity, which would in turn attenuate LCFA synthesis and subsequently reduce FA metabolism-dependent tumor growth, which would precipitate tumor stasis (Fig. 3c). This could provide a satisfactory explanation for the clinical observation that IDH1 mutated gliomas have a better clinical prognosis compared to their wild-type counterparts [39]. However, the mechanism underlying the loss of tumor suppression by IDH2 mutations remains currently opaque since the mitochondrial seat of

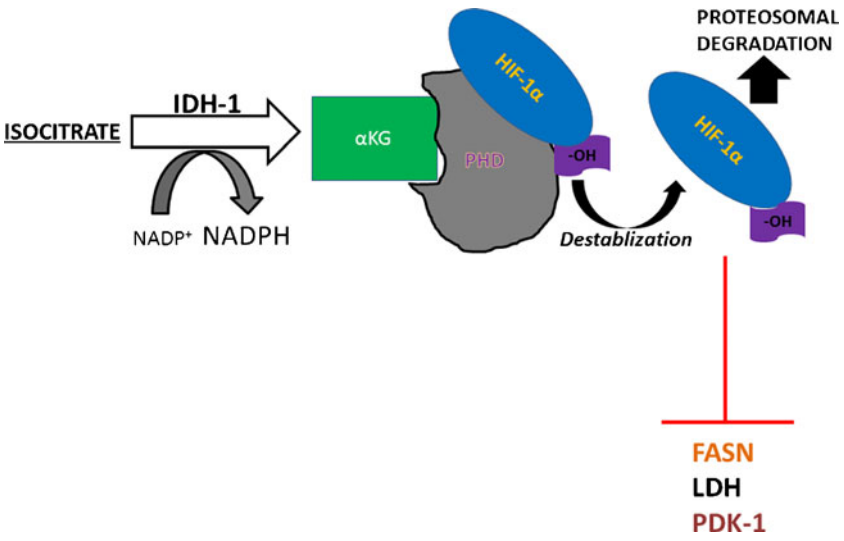

Fig. 2 Wild-type isocitrate dehydrogenase (*IDH*)-*1* protein mediates the destabilization of HIF-1α in normoxia and thus suppresses the expression of HIF-1α-dependent metabolic enzymes involved in both fatty acid synthesis (*FASN*), the repression of pyruvate oxidation (*PDK-1*) and glycolysis (*LDH*)

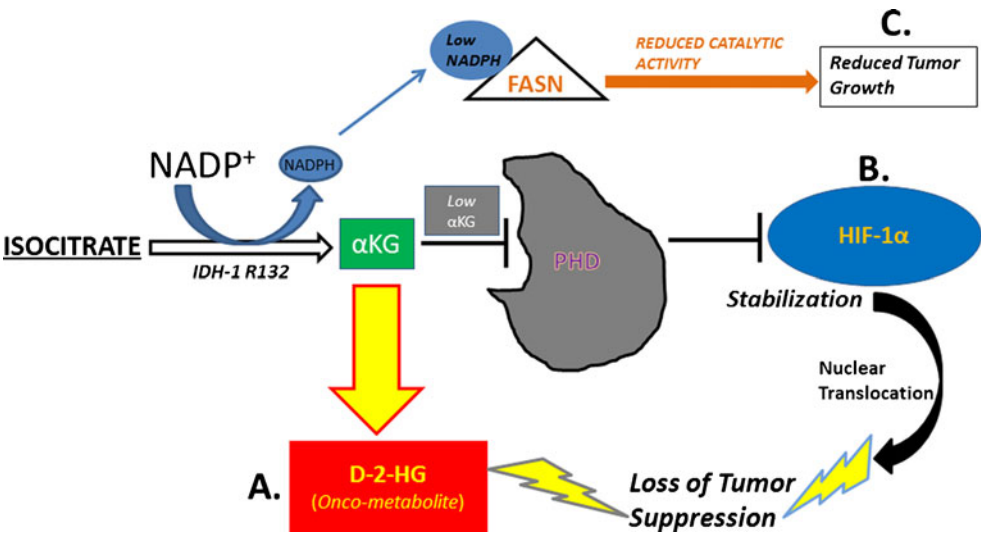

Fig. 3 IDH-1 R132-dependent loss of tumor suppression contributing to the oncogenesis of human gliomas by: *a* D-2-HG (D-2-hydroxyglutarate) oncometabolite production and *b* HIF-1α protein stabilization. *c* Reduced NADPH-dependent FASN activity causing attenuated tumor growth

IDH2 mutated proteins would not have any effect on HIF-1α stabilization, and it is not apparent how mitochondrial accumulation of D-2-HG and/or depletion of mitochondrial NADPH could trigger derepression of oncogenesis, unless each or both of these factors perturbed TCA cycle metabolism, akin to the cellular phenotypic effects of germline fumarate hydratase or succinate dehydrogenase (SDH) mutations [6].

Unlike the differential effect of HIF-1α and HIF-2α on glucose metabolism in xenograft cancer models [42], it remains to be conclusively determined whether these two HIFα isoforms have contrasting effects on FA synthesis and metabolism, although in a premalignant nonalcoholic fatty liver disease *in vivo* model system, HIF-2α has been demonstrated to regulate the expression of a lipid storage protein, adipose differentiation-related protein (ADFP), which could suggest that ADFP is an early and initiating event in the pathogenesis of HIF-2α-mediated steatosis [43] and therefore could be an important initiating factor in hepatocellular carcinogenesis. In contrast, given that high levels of expression of either ADFP or MAG are associated with low-grade differentiation of a variety of solid tumors [19, 44], the genetic reprogramming of cancer cells to express enzymes which catabolize lipid storage proteins might be a necessary early adaptation to maintain high levels of LCFA synthesis that would be required to assist cellular biosynthetic processes for high-grade transformation.

In this light, LCFAs *per se*, rather than their cognate lipid storage proteins, might also be involved in the evasion of apoptosis and in the posttranslational modification of oncogenic proteins in premalignant cells. For instance, exogenous treatment of mouse embryonic fibroblasts with saturated LCFAs attenuates the activation of the ATR-p53 pathway to genotoxic insults [45], thus suppressing apoptosis and augmenting cell survival. This, or a similar modulating effect on DNA repair, could also be hypothesized to be involved in the development of FASN-associated anthracycline resistance in human invasive breast cancer cells [46]. Furthermore, posttranslational palmitoylation of oncogenic proteins, such as mutated RAS, SRC family tyrosine kinase receptors, heregulin (HER2) receptor [47], and the cell invasion regulator membrane-type 1 matrix metalloproteinase [48], is essential for their individual localization and functions at the plasma membrane.

7 Fatty acid β-oxidation

In humans, the subcellular seat for β-oxidation switches from peroxisomes to mitochondria with the postnatal availability of LCFAs in maternal breast milk and β-oxidation continues to remain *in situ* within the mitochondria of cancer cells [49, 50]. The first β-oxidation reaction is the cytoplasmic metabolism of LCFA to their respective acyl-CoA derivatives by acyl-CoA synthase (ACS) isoforms. There are 26 genes encoding ACSs that have discriminatory affinities for their ability to activate short-, medium-, long- and very long-chain fatty acids, respectively [51]. For instance, very long-chain-3 ACS (ACSVL3) is overexpressed in glioblastomas in comparison to low-grade gliomas, such that attenuation of ACSVL3 expression in glioblastoma cells *in vitro* and *in vivo* is associated with reduced cell proliferation and tumor growth, respectively [22, 52]. ACSVL3 is regulated upstream by EGFR tyrosine kinase receptors [22] in tumors expressing either wild-type overexpressed (amplified) or mutated EGFRvIII proteins, the latter of which might represent a glioblastoma-specific biomarker of β-oxidation-dependent tumor growth that could also represent a tractable therapeutic target in glioblastoma [53].

Long-chain acyl-CoAs are subsequently esterified to their L-carnitine derivatives by CPT-I on the surface of the outer mitochondrial membrane before mitochondrial import

for β-oxidation [50], such that the principal long-chain acyl-CoA, palmitoyl-CoA (PALMCoA), is metabolized by CPT-I to palmitoylcarnitine. In normal untransformed cells when ATP reserves are high, and AMPK activation is suppressed, elevated levels of malonyl-CoA inhibit CPT-I, thus preventing the β-oxidation of newly formed LCFAs [50]. Conversely during starvation, when ATP levels are low and AMPK is activated, ACC-dependent malonyl-CoA levels fall, releasing the inhibition on CPT-I which thus facilitates the mitochondrial entry of LCFAs for β-oxidation. The latter is reminiscent of the cancer cell microenviroment, but in this case, β-oxidation-derived acetyl-coA is used principally to suppress pyruvate oxidation, thus augmenting glycolysis, and/or supporting anapleurosis through increased β-oxidation, a process which is augmented by increased AMPK activation which triggers increased mitochondrial biogenesis and further β-oxidation. Moreover, it could be hypothesized that both MAGL and AMPK cooperate in high-grade tumors to maintain a high level of LCFA substrate flux through β-oxidation greater than the rates of reaction involved in LCFA synthesis *per se*.

PALMCoA is subsequently metabolized by four enzymatic reactions to produce acetyl-CoA. This utilizes a quartet of cornerstone β-oxidation enzymes that reside on different mitochondrial substructures [54], which *in toto* suppresses glucose oxidation by repressing PDH activity, thus preventing the mitochondrial metabolism of pyruvate (Fig. 4). The first β-oxidation enzyme, acyl-CoA dehydrogenase (ACD), has three principal isoforms—very long-chain ACD (ACAD9), medium-chain ACD, and short-chain ACD [55, 56]. Whether the expression of these three ACD isoforms is dependent on the histological type of a cancer or whether morphological differentiation is regulated by the balance of expression of different isoforms within a particular cancer is not known, but somatic mutations in neither gene have been identified to date in human cancers, although germline mutations can cause severely detrimental clinical metabolic phenotypes in affected humans [56].

The last three proteins involved in the β-oxidation enzyme quartet are known as the trifunctional protein (TFP) [57], [58]. TFPα comprises the first two of the three proteins—hydroxyacyl-CoA:NAD^+ oxidoreductase (HAD) and enoyl-CoA hydratase (ECH) [58], which are encoded by the HADHA gene [57]. TFPβ is encoded by HADHB and encodes acetyl-CoA acyltransferase (ACAA2; ketoacyl-CoA thiolase) [57, 58]—the final enzyme required for β-oxidation to generate acetyl-CoA. Given that no somatic mutations in HADHA and/or HADHB have been reported to date in human tumors (COSMIC mutation database search [59]; accessed 13 February 2012), and that they are both spatially orientated in a "head-to-head" configuration in chromosome 2p, cancer cells may have evolved mechanisms to evade permissive genotoxic damage at these loci, particularly since germline mutations of both genes (complete TFP deficiency), or of mutated HADHB alone [56], are associated with a severe metabolic clinical phenotype.

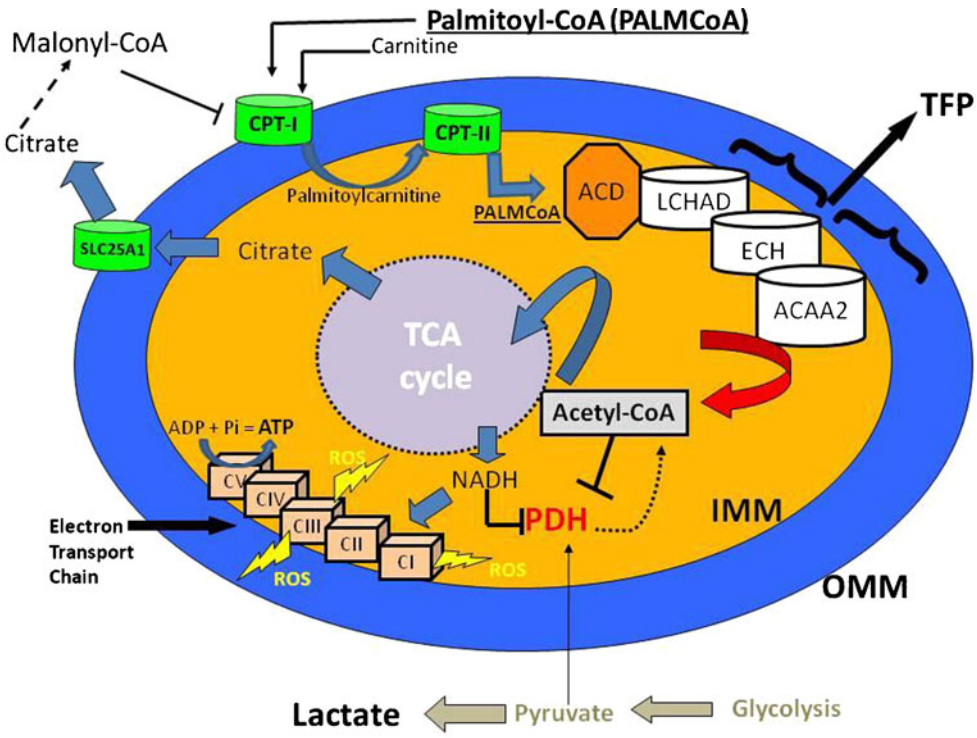

Fig. 4 Mitochondrial fatty acid β-oxidation suppresses pyruvate-mediated glucose oxidation by repression of PDH (*IMM* inner mitochondrial membrane, *OMM* outer mitochondrial membrane, *ACD* acetyl-CoA dehydrogenase, *LCHAD* long-chain hydroxyacyl-CoA:NAD^+ oxidoreductase, *ECH* enoyl-CoA hydratase, *ACAA2* acetyl-CoA acyltransferase, *PDH* pyruvate dehydrogenase, *ROS* reactive oxygen species, *UCP* uncoupling proteins)

8 Cellular mechanisms to mitigate mitochondrial lipotoxicity

Non-esterfied LCFAs that have not been transported into mitochondria for β-oxidation are highly reactive with cellular ROS. The resulting lipid peroxidation species, such as 4-hydroxynoneal, are highly damaging to mitochondrial (mt) DNA, mtDNA repair enzymes and TCA cycle enzymes, such as m-actinose [60]. Untransformed skeletal myocytes avoid the deleterious effects of lipid peroxidation by upregulating the expression of inner mitochondrial membrane (IMM) "uncoupling" proteins (UCPs), such as UCP3, which also acts as a mitochondrial lipid anion exporter [61].

The UCPs (UCPs 1–5) dissipate ("short circuit") the mitochondrial proton gradient (ΔΨM), thus "de-energizing" mitochondria and attenuating ATP production. In human leukemia cells, UCP-mediated metabolic reprogramming prevents the utilization of pyruvate for oxidation such that pyruvate can no longer enter mitochondria and is therefore metabolized in the cytoplasm by LDH to lactate [62, 63], constituting an alternative mechanism for the induction of the Warburg effect.

In both normal cells and human leukemia cells, UCPs are bioenergetic/biosynthesis adapter molecules that shift the carbon skeleton for mitochondrial oxidation from glucose to LCFAs [62], by increasing the resistance of mitochondria to ROS, particularly since β-oxidation produces more reactive intermediate products than pyruvate oxidation. Since this can only be achieved by reducing ΔΨM, ATP levels are reduced, and the apoptotic potential of β-oxidation-dependent cells is reduced too, which is a contributing factor to the development of resistance to cytotoxic agents in FA metabolism-dependent solid tumor cells [64]. Therefore, to facilitate both β-oxidation and mitochondrial integrity to ensure cell survival, the cancer cell attenuates ATP from β-oxidation in a UCP-dependent manner. This leads to an overall deficit in cellular ATP production which is probably filled by generating ATP from the aerobic glycolysis which is triggered by β-oxidation-mediated suppression of PDH (see Fig. 4), as well as from the consequent activation of AMPK by increasing AMP that increases the flux of LCFAs through β-oxidation, thus generating higher ATP levels/time. Therefore, it is not surprising that both palmitic acid and mitochondrial superoxide (O_2^{-}) are UCP activators

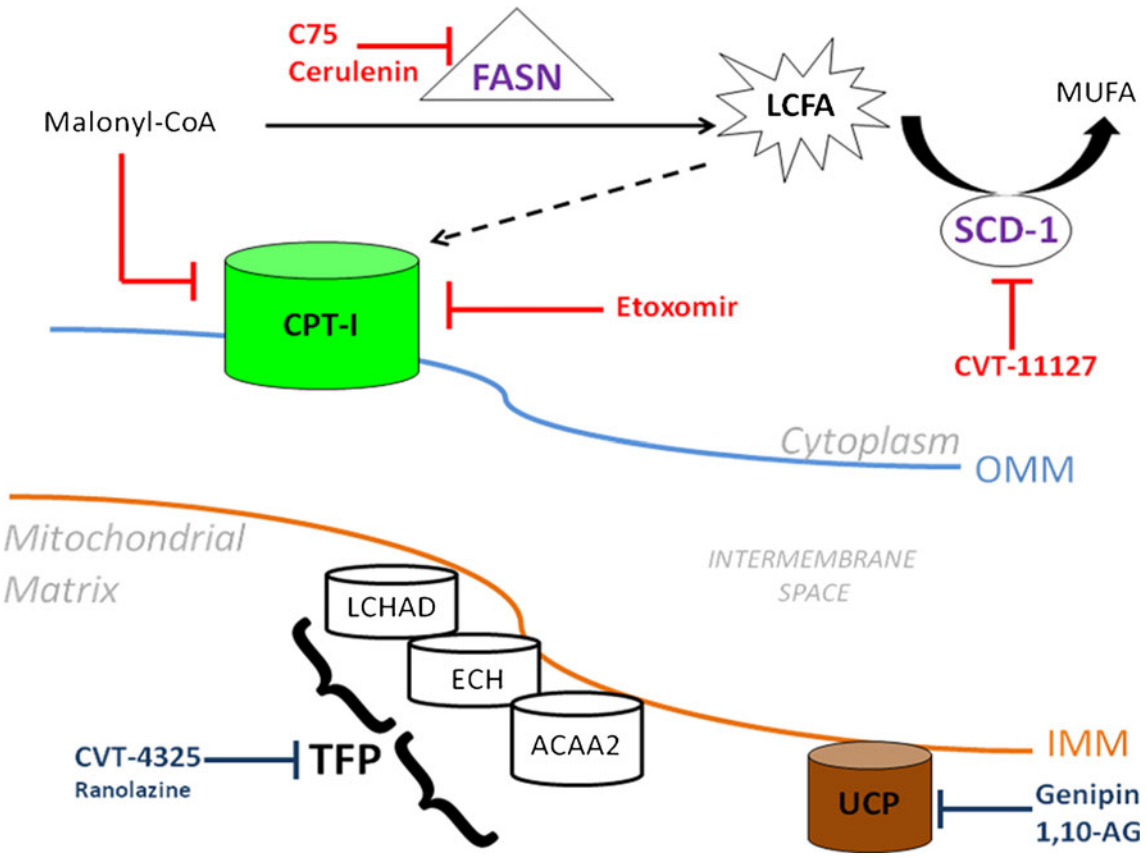

Fig. 5 Specific inhibitors of fatty acid metabolism in human cancer cells. Inhibitors of *de novo* fatty acid synthesis are highlighted in *red*: C75 and cerulenin = FAS inhibitors; CVT-11127=SCD-1 inhibitor. Inhibitors of fatty acid mitochondrial metabolism in *blue*: CVT-4325 and Ranolazine = TFP inhibitors; Genipin and 1,10-AG = UCP inhibitors which would be predicted to cause cell death by preventing the attenuation of ROS in cells reliant on β-oxidation

[65], but it is noteworthy that mesenchymal stromal feeder cells seem to be important regulators of UCP expression in human leukemia cells too [61, 62], which alludes to the possibility that exogenous signaling from nearby host cells might also regulate cancer cell UCP expression. Interestingly, in normal human fibroblasts, upregulation of UCP2 expression causes a reprogramming of nuclear encoded mitochondrial gene expression which involves the upregulation of mitochondrial proteins involved in both mitochondrial biogenesis (PGC-1α) and β-oxidation (CPT-I) [66]. Whether a similar nuclear reprogramming occurs in cancer cells with high UCP2 levels is not known, but such a mechanism could also be predicted to mitigate the cellular ATP deficit in β-oxidation/UCP-dependent cells.

9 Fatty acid metabolism and regulation of apoptosis

Fatty acid synthesis and β-oxidation in human leukemic cells seem to be intimately linked to apoptosis since both Orlistat-induced FAS inhibition and Etomoxir-induced CPT-I inhibition cause apoptosis, respectively [63]. Unexpectedly, Etomoxir in leukemia cells seems not to trigger apoptosis by increasing mitochondrial ROS production from the derepression of pyruvate oxidation, but in fact possibly by CPT-I-mediated effects at the outer mitochondrial membrane. In normal cells *in vitro*, CPT-I is known to co-localize with both the pro-apoptotic protein tBID [67] and the anti-apoptotic protein BCL-2 [68]; whether the binding of Etomoxir to CPT-I affected the balance between its associations with pro- and anti-apoptotic proteins at the mitochondrial membrane and thus triggering apoptosis in leukemic cells remains to be investigated. Whether inhibition of other β-oxidation enzymes has similar effects could be investigated by using chemical inhibitors against TFP, such as CVT-4325 [69] or Ranolazine, or against UCPs using Genipin or 1,10-AG [70] (Fig. 5). Interestingly, the converse may also be the case since pro-apoptotic proteins, such as tBid, could regulate β-oxidation by binding to and inhibiting CPT-I-mediated LCFA mitochondrial import [67].

Furthermore, Etomoxir-mediated apoptosis in human leukemic cells has been demonstrated to be potentiated by small molecule inhibitors targeted against the cell's anti-apoptotic proteins, such that ABT-737 (anti-BCL-2) and Nutlin-3A (MDM2 antagonist) augmented apoptosis in proliferating cells [63], as well as in *ex vivo* quiescent primary leukemia cells from patients when also combined with ABT-737, as well as the nucleoside analog Ara-C [63]. This would suggest that although FA metabolism and apoptotic potential might be inextricably linked in leukemia cells, it is not cell cycle dependent; whether this is the case in solid tumors remains to be investigated. However, it is noteworthy that another β-oxidation enzyme ACAA2 rescues solid tumor cells from the pro-apoptotic effects of exogenously overexpressing the atypical BH3-domain BCL-2 protein, BNIP3, possibly by its association together on the inner mitochondrial membrane [71]. Therefore, it seems not unreasonable to postulate that β-oxidation enzymes might have constitutive anti-apoptotic functions that are mediated by their interactions with mitochondrial pro-apoptotic proteins.

10 Conclusion and clinical translational opportunities

Cancer cells demonstrate an *a priori* reprogramming of fatty acid metabolism, which in concert with an upregulation of glycolytic proteins can cause the induction and maintenance of a Warburg-*ian* effect in cancer cells. Non-glucose cancer cell metabolism is a recent discovery which has revolutionized our understanding of cancer biology [1]. We are beginning to understand that glucose is not the only carbon skeleton for cellular biosynthesis and ATP production [1, 10, 72] and that certain types of cancer cells seem to have a greater "addiction" to fatty acids than to glucose, potentially providing therapeutic opportunities for tumor-specific therapeutic interventions. Furthermore, given that the development of metastasis in cancer patients is frequently accompanied by significant changes in whole body organ metabolism, careful preclinical testing of compounds that perturb FA metabolism in preclinical models would be essential, such that a systems biology approach using *in silico* computational models in addition to noninvasive functional imaging techniques in xenograft models would be required. Furthermore, *in vivo* preclinical models could also be used to determine whether the metabolic signature of primary tumors differs from their metastasis and whether metabolic switching of carbon skeletons (*e.g.*, from glucose to fatty acids), or upregulation of FA metabolism *per se*, accompanies tumor progression. Similarly, the contribution of FA metabolism in premalignant cells to tumorigenesis remains to be elucidated.

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