Molecular Pathways: Tumor Cells Co-opt the Brain-Specific Metabolism Gene \(CPT1C\) to Promote Survival

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Abstract

The metabolic adaptations of cancer cells are receiving renewed attention as potential targets for therapeutic exploitation. Recent work has highlighted the importance of fatty acid catabolism through \(\beta\)-oxidation to cellular energy homeostasis. In this article, we describe recent preclinical studies suggesting that a gene usually expressed only in the brain, carnitine palmitoyltransferase (\(CPT1C\)), promotes cancer cell survival and tumor growth. \(CPT1C\) confers rapamycin resistance on breast cancer cells, indicating that this gene may act in a pathway parallel to \(mTOR\)-enhanced glycolysis. Because of \(CPT1C\)'s normally brain-restricted expression and the inability of most drugs to pass the blood–brain barrier, \(CPT1C\) may be an ideal candidate for specific small-molecule inhibition. We further speculate that concurrent targeting of \(CPT1C\) activity and glycolysis in tumor cells could be a highly effective anticancer approach. *Clin Cancer Res; 18(21); 5850–5. ©2012 AACR.*

Background

**Cancer cell metabolism**

Tumor cells exhibit unique metabolic adaptations that are increasingly viewed as potential targets for novel and specific cancer therapies. A well-known example of such an adaptation is the long-established Warburg effect in which tumor cells exhibit defective oxidative phosphorylation (OXPHOS; refs. 1, 2). His observation of this phenomenon led Warburg to propose that mitochondrial dysfunction is the primary cause of cancer. Subsequent studies have shown that tumor cells exhibit several additional metabolic differences from normal cells, including an increased rate of glucose uptake that feeds a high rate of glycolysis, a more oxidative intracellular environment, and an ability to tolerate hypoxic conditions (3–5). Indeed, these properties are collectively now considered an "emerging hallmark" of cancer, despite their recognition decades ago (6). This revisited recognition is based on the potential to use the metabolic aberrations as targets for therapeutic exploitation, which, with the advent of specific small-molecule inhibitors (7), is now practical.

Until recently, the cancer research community held a conventional belief that increased glycolysis in a tumor cell compensated for the cell’s loss of ATP production due to defective OXPHOS. Although almost certainly part of the explanation, this scenario is complicated by the fact that many of the metabolites of glycolysis are diverted from glycolytic ATP production by the cancer cell–specific enzyme pyruvate kinase M2 (PKM2; ref. 8). Rather than generating ATP, these metabolites are processed through the pentose phosphate pathway (PPP) to produce additional metabolites, such as ribose 5-phosphate and, perhaps more importantly, reduced NADPH, which then regenerates the reduced form of the antioxidant tripeptide glutathione (9). This antioxidant aspect of cancer metabolism is believed to be crucial for counteracting the reactive oxygen species (ROS) arising due to oncogene activation and the heightened proliferation of these cells (10). The generation of these antioxidants through the PPP, however, comes at the cost of ATP production. Because the demand for ATP in cancer cells is enhanced, it is likely that additional dramatic metabolic adaptations occur in cancer cells to provide the energy necessary for survival and proliferation (11).

Recent work has highlighted the importance of fatty acid catabolism through \(\beta\)-oxidation to cellular energy homeostasis in tumor cells. This route not only supplies ATP but also provides an additional opportunity to generate reduced nucleotides. \(\beta\)-Oxidation is the chemical breakdown of fatty acids that uses NAD\(^+\) and FAD\(^2+\) precursors to produce one NADH and one FADH\(_2\) for every two carbons removed (12). These reduced products can then provide electrons for transport in any residual OXPHOS-mediated ATP generation in a tumor cell, or may act as antioxidants. However, it remains unclear if and how glutathione reduction in the mitochondria might affect oxidation potential in the cytosol (13). Regardless, the accumulating evidence suggests that some cancers use fatty acid oxidation (FAO) as an important energy source for survival and proliferation (14–16). In Fig. 1A, we outline our model for tumor cell...
metabolism in which parallel pathways involved in the regulation of fatty acid and glucose metabolism are proposed to provide ATP and antioxidants to cancer cells.

**Clinical targeting of cancer metabolism**

The increased attention provided to the distinctive characteristics of tumor cell metabolism has led to various clinical interventions (for a recent review, please see ref. 7). Some of these treatments have focused on disrupting the increased glycolysis in cancer cells. For example, the substrate analog 2-deoxyglucose (2-DG) inhibits glycolysis at several steps and is an effective cytotoxic agent (17), suggesting its use as a component of a combination therapy. Metformin is a useful antiglucose drug that lowers blood glucose by inhibiting ATP production in mitochondria (18, 19). This inhibition stimulates AMP-dependent kinase (AMPK), which in turn leads to a blockage of gluconeogenesis in the liver. Independent of this systemic effect, metformin has anticancer properties at high doses (20, 21). Rapamycin is one of several inhibitors of mTOR complex 1 (mTORC1; ref. 22), which is an important effector downstream of phosphoinositide 3-kinase (PI3K). These cytostatic agents function in part by reducing glucose transport into the cell, thus limiting glycolysis (23). While some success in halting tumor cell growth has been achieved using these agents (22), they have not proved to be a clinical panacea (24). Additional targets are under investigation, including those that target fatty acid catabolism.

**Functions of CPT1 enzymes**

The carnitine palmitoyltransferase 1 (CPT1) family of proteins plays a critical role in regulation of FAO in normal cells (25). These enzymes are present on the cytosolic surface of mitochondria and catalyze the production of acylcarnitine from acyl-CoA, thereby facilitating lipid transport through the mitochondrial membranes. Acyl carnitine production represents a critical regulatory step before β-oxidation because the CPT1 enzymes can be inhibited by malonyl-CoA, a terminal metabolite of the β-oxidation of many acyl-CoA molecules. Malonyl-CoA, as a fatty acid precursor, simultaneously drives fatty acid biosynthesis and impairs fatty acid catabolism, thus regulating the balance of intracellular fatty acids (26).

The CPT1 family is composed of 3 proteins that show tissue-specific distribution. CPT1A was first identified in the liver but is in fact broadly expressed (25). Mutations of the CPT1A gene have been implicated in hereditary disorders. In contrast, CPT1B expression is restricted to muscles, and CPT1C is a brain-specific isoform (25). Unlike CPT1A and CPT1B, comparatively little is known about the regulation and function of CPT1C. Indeed, it has not yet been shown that CPT1C has carnitine-transferase activity in biochemical assays (27). Instead, the existing evidence suggests that CPT1C signaling integrates into molecular pathways in the hypothalamus that regulate food intake (27, 28) and systemic energy use (29).

**CPT1C promotes tumor growth and drug resistance**

A role for CPT1C in tumor growth was first revealed by Zaugg and colleagues (30) in a study showing that CPT1C expression in an extensive panel of breast cancer xenografts correlated inversely with mTOR activation and rapamycin sensitivity. The xenograft tumors were analyzed for CPT1C mRNA expression, mTOR activation status, and rapamycin sensitivity. Not surprisingly, tumors with increased mTOR activation were also more likely to be sensitive to rapamycin. What was surprising was that the rapamycin-sensitive tumors consistently displayed lower CPT1C mRNA expression, whereas rapamycin-resistant tumors were more likely to show high levels of CPT1C mRNA. This was not the case for either CPT1A or CPT1B mRNAs. Indeed, among the thousands of genes analyzed, CPT1C mRNA expression was most strongly correlated with mTOR activation. The absence of any known regulatory interaction between mTOR and CPT1C suggested that mTORC1 and CPT1C act in parallel pathways in cancer cells to achieve a similar outcome. Given the known activities of CPT1 proteins in catabolic energy generation in normal cells, it was theorized that CPT1C might be supplementing the high energy needs of cancer cells via FAO, and that cancers lacking CPT1C could not take advantage of this supplementation, and thus were sensitive to rapamycin. Consistent with this hypothesis, CPT1C overexpression in vitro in a breast cancer cell line increased ATP synthesis as well as FAO. Conversely, siRNA-mediated CPT1C depletion in vitro cooperated with rapamycin and 2-DG to inhibit cancer cell growth (30). Intriguingly, CPT1C-deficient embryonic stem cells and CPT1C-depleted cancer cells were also more sensitive to other metabolic stresses, including culture under conditions of low glucose or hypoxia (30). This inverse relationship between CPT1C expression and rapamycin sensitivity implied that CPT1C might be a possible novel target for a combined therapy that would disrupt cancer cell metabolism and kill tumor cells that had become resistant to drugs targeting the PI3K pathway.

Of course, for CPT1C to reach its potential as an anticancer target, this normally brain-specific isoform would have to be expressed in a broad range of cancers at relatively high frequency. The work of Zaugg and colleagues, which had already shown the unusual expression of CPT1C in many breast cancers, also showed that CPT1C mRNA was overexpressed in 13 of 16 lung cancers as compared with levels in paired normal tissues (30). Our most recent examinations of CPT1C mRNA expression in a wide array of tumor types have shown that CPT1C is highly expressed in brain cancers and several sarcomas (Fig. 1B). Interestingly, hematopoietic malignancies have the lowest levels of CPT1C mRNA, perhaps reflecting their greater access to nutrients in the bloodstream. The fact that aberrant CPT1C expression occurs in a broad range of tumor types suggests that CPT1C inhibition may hold promise as a therapy for a significant proportion of cancers.
CPT1C regulation

Although CPT1C expression is restricted to the brain in healthy individuals, it functions as a stress-responsive gene under a variety of conditions. Specifically, CPT1C mRNA was found to be upregulated in cell lines from several different tissues as well as in mice in vivo following exposure to any one of a number of p53-activating stresses. Reporter assays showed that CPT1C is a direct transcriptional target of p53 and is the only stress-responsive member of the CPT1 family (30). Our finding that p53 influences a metabolic regulator in normal cells is not without precedent. Maddocks and Vousden (31) recently reviewed the growing literature on the role of p53 in metabolic regulation. In normal cells, p53 functions to suppress glycolysis and increase FAO and OXPHOS. Thus, perhaps in a manner parallel to how it responds to DNA damage, p53 induces the expression of metabolic regulators (as it would DNA repair factors) and only signals cell death if the crisis cannot be resolved. However, in lung cancer, CPT1C overexpression is likely not p53 dependent, as p53 is either mutated or present at very low levels in these malignancies (30). Thus, a p53-independent mechanism, the details of which are still unclear, seems to regulate CPT1C in cancer cells.

Our evidence also points to positive regulation of CPT1C expression by AMPK. First, metformin treatment in vitro upregulates CPT1C mRNA (30). Second, primary fibroblasts from AMPK-deficient mice fail to upregulate CPT1C when exposed to low glucose and/or hypoxic conditions in vitro. Moreover, when CPT1C was depleted in tumorigenic cells and xenografted them into mice, the malignancies that developed were insensitive to metformin treatment (30). More recent data suggest that AMPK-mediated enhancement of CPT1C mRNA is mainly p53 dependent (32), but that at least some of the increase in CPT1C protein associated with AMPK activation is p53 independent. This p53-independent regulation may be related to the finding that AMPK exerts control over CPT1C translation through the CPT1C 5′UTR (33).

Clinical–Translational Advances

Effective metabolic targeting

Despite initial optimism, the problem of cancer drug resistance has not been relieved by the emphasis of the last 20 years on more specific oncogene targeting. The genetic and epigenetic heterogeneity in a tumor allow it to rapidly mutate, adapt, and inexorably grow. As a result, the last few years have seen a shift away from drugs designed to inhibit oncogenic drivers of cancer and toward agents that can block factors enabling the growth and/or survival of tumor cells. The targeting of tumor angiogenesis (34, 35), inflammation (36, 37), and immune evasion (38) are all receiving increasing attention in this respect. The metabolic adaptations displayed by cancer cells are also being viewed with fresh eyes and evaluated for their potential as drug targets. Thus far, the results of monotherapies with agents blocking metabolic pathways have been disappointing, either because of high-dose toxicity, as for 2-DG (39, 40), or because of drug resistance, as for mTOR inhibitors (24). Combination therapy is therefore a superior strategy for circumventing these obstacles. As mentioned earlier, the work on CPT1C has led us to devise a model of cancer cell metabolism in which FAO and glycolysis function in parallel to provide the cell with energy and antioxidants (refer to Fig. 1A). We believe that a combined treatment that simultaneously targets FAO and glycolysis and removes these supports of cancer cell survival may be a highly effective therapeutic approach.

CPT1C represents a particularly attractive target to this end. CPT1 proteins are enzymes and as such should be relatively easily inhibited by small molecules. Etomoxir is a small-molecule inhibitor that binds to CPT1 proteins and inhibits the enzymatic activities of CPT1A and CPT1B in vitro. However, tests of etomoxir as a treatment of diabetes or congestive heart failure have yielded disappointing results (41, 42). Obviously, because no substrate has been identified for CPT1C and a biochemical assay is thus lacking, progress toward identifying an agent that specifically targets CPT1C functions has been slow. The evidence that CPT1C expression allows a tumor cell to overcome metabolic stress should spur researchers to redouble their efforts to find an effective CPT1C inhibitor. A CPT1C-specific inhibitor, as opposed to a pan-CPT1 inhibitor, would be particularly useful as an anticancer agent because CPT1C expression is restricted to the brain in an otherwise healthy person. Because most small-molecule drugs cannot easily pass the blood–brain barrier (43), a CPT1C-specific inhibitor would likely reduce the chance of undesired side effects, and thus be a safer alternative to approaches that more broadly inhibit FAO.

Why CPT1C?

With respect to FAO and cancer, an intriguing question remains, why would tumors activate a brain-specific gene
for fulfilling their metabolic needs, particularly when more broadly expressed family members, namely CPT1A and CPT1B, are also available but apparently not activated in cancer cells (44). Presumably, both CPT1A and CPT1B can also increase FAO (45, 46), so it stands to reason that CPT1C upregulation must provide a special activity that spurs tumor growth. The simplest explanation would be that CPT1C acts on different substrates than CPT1A and CPT1B. For example, selective fatty acid degradation mediated by CPT1C activation might provide an advantage for anabolic tumor cell proliferation. The finding that long-chain polyunsaturated fatty acids, such as arachidonic acid, accumulate in CPT1C-deficient embryonic stem cells also suggests that CPT1C has distinct substrate specificities (30).

Another possibility is that CPT1C may perform additional roles beyond its involvement in FAO. Work by the Casals laboratory has furnished evidence that CPT1C is localized to the endoplasmic reticulum (ER) membrane in neurons (47), in contrast to the other CPT1 proteins, which are positioned on the exterior surface of the mitochondria. The Casals group has also shown that CPT1C functions to increase intracellular ceramide levels (47, 48), again implying a difference in substrate specificity. This latter observation is puzzling, however, because ceramide is considered a proapoptotic lipid and thus an unlikely substance that a cancer cell would accumulate (49). Moreover, the localization of CPT1C on the ER membrane is difficult to reconcile with the defect in mitochondrial morphology observed in CPT1C-deficient embryonic stem cells (30). Finally, the observed roles of CPT1C in appetite control and systemic energy expenditure (50) suggest that this enigmatic protein may affect additional pathways that have yet to be identified.

CPT1C is the only CPT1 family member that is regulated by p53 (30, 51), but how this property fits into tumor cell expression of CPT1C is a mystery. Perhaps the activation of CPT1C by p53 in normal cells subjected to metabolic stress means that the CPT1C gene is more susceptible to epigenetic modification upon loss of p53 function. Alternatively, CPT1C may promote FAO better than either CPT1A or CPT1B in cells attempting to grow under stress conditions, such as in an oxidative environment. These issues remain to be clarified. Practically speaking, however, evidence in preclinical studies (30) suggests that the targeting of CPT1C, particularly in combination with mTOR inhibitors, offers an excellent opportunity to disrupt cancer cell metabolism and thereby slowing tumor growth.

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No potential conflicts of interest were disclosed.

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Conception and design: T.W. Mak
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