REVIEW

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Balancing biosynthesis and bioenergetics: metabolic programs in oncogenesis

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Abstract

Cancer biologists' search for new chemotherapy targets is reinvigorating the study of how cancer cell metabolism determines both oncogenic potential and chemotherapeutic responses. Oncogenic metabolic programs support the bioenergetics associated with resistance to programed cell death and provide biosynthetic building blocks for cell growth and mitogenesis. Both signal transduction pathway activation and direct mutations in key metabolic enzymes can activate the metabolic programs that support cancer cell growth. Cancer-associated metabolic programs include glycolysis, glutamine oxidation, and fatty acid metabolism. Recent observations are revealing the regulatory mechanisms that activate cancer-associated metabolism, and the competitive advantages provided to transformed cells by their metabolic programs. In this study, we review recent results illustrating the mechanisms and functional impact of each of these oncogenic metabolic programs in cancer cell growth and survival.

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Introduction

Cancer-associated functional changes have been conceptualized as the hallmarks of transformed cells, including unrestrained cell growth, autonomous mitogenesis, and resistance to apoptosis. Perhaps to satisfy the metabolic demands of these processes, cell transformation is also frequently associated with elevated cellular metabolism. Recently, newly uncovered links between oncogene-induced signal transduction networks and metabolic enzymes have rekindled interest in targeting cancer cell metabolism with chemotherapeutics. These advances raise fundamental questions in carcinogenesis: what determines the metabolic portfolio of cell metabolism in cancer cells? What is the functional benefit of specific metabolic programs to cancer cell growth and survival? In this review, we aim to describe metabolic alterations that are associated with cell transformation (Fig. 1), the mechanisms linking metabolism to oncogenes, and the functional impact of altered metabolism for cancer growth and chemotherapy.

Glycolysis: the metabolic program for growth and survival

Glycolysis in cancer cells

Glycolysis is the metabolic program of choice for cells that are actively engaged in cell growth and mitogenesis. A number of models have been proposed to explain the preference for glycolytic metabolism in transformed cells: i) bioenergetic demand due to mitochondrial defect – cancer cells have no other choice, because mitochondrial oxidative phosphorylation has been compromised by cancer-causing mutations; ii) biosynthesis – glycolytic metabolism generates biochemical intermediates that are useful for the synthesis of macromolecules for cell proliferation, such as fatty acids and nucleotides; iii) temporal programing – metabolic programs are utilized by cells according to patterns established by oscillating control mechanisms in the cell, such as cell cycle. Recent advances supporting each of these perspectives and their implications for cancer therapy are discussed below.

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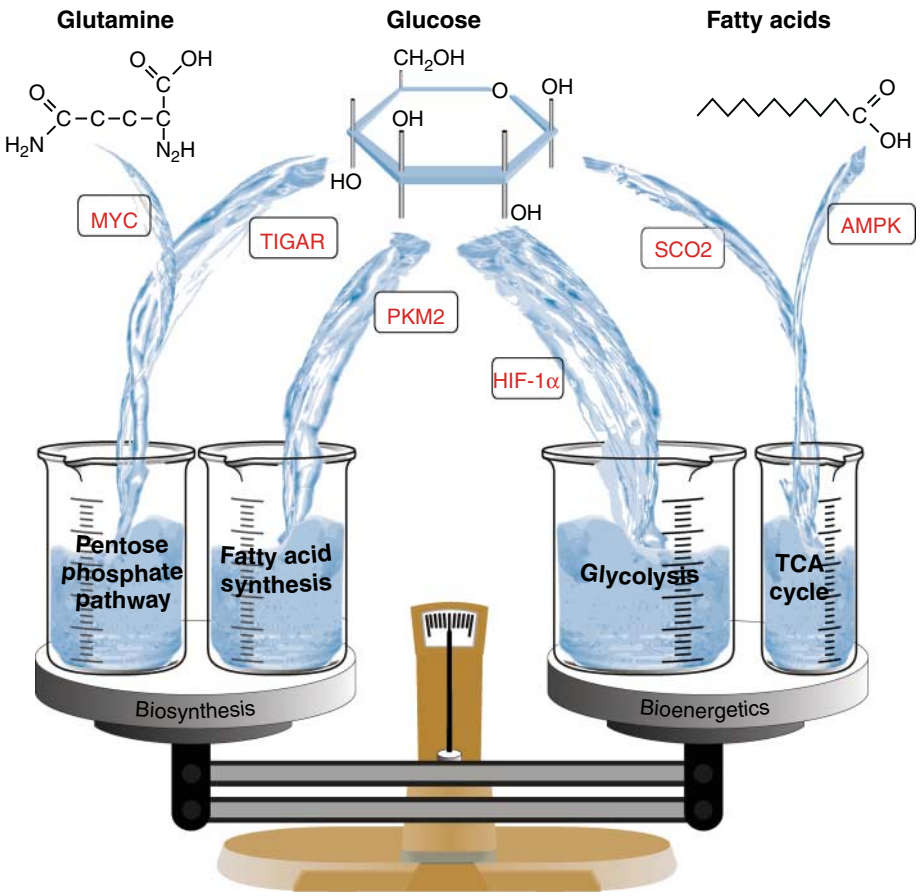

Figure 1 The bioenergetic and biosynthetic requirements of cancer cells are balanced by regulating the flux of pathways that metabolize glucose, glutamine, or fatty acids. Key regulatory mechanisms that determine the flux from each carbon source into the pathways are highlighted. The model shows HIF-1α and AMPK driving increased bioenergetic input derived from glucose and fatty acids. PKM2, TIGAR, or c-Myc can promote the flux of glucose or glutamine into biosynthetic pathways. SCO2 promotes glucose oxidation via the TCA cycle.

Bioenergetic demand

In his pioneering work describing decreased oxidative metabolism coupled with increased glycolysis in cancerous tissues, Warburg *et al*. (1931) observed that the acquisition of glycolytic metabolism is irreversible, as transformed cells rarely revert to an oxidative form of metabolism. This is in contrast to embryonic tissues, which exhibit high rates of glycolysis but eventually switch to oxidative metabolism as proliferation ceases and cells differentiate. One possible explanation for the inability of cancer cells to engage oxidative metabolism is the acquisition of mutations in key enzymes, in effect delivering a 'metabolic injury' that irrevocably alters metabolic flux. As genomic data from cancer cells accumulate, new oncogenic mutations in metabolic enzymes are emerging, thus demonstrating the critical impact of metabolic pathways in maintaining cellular homeostasis.

Mutations that disrupt mitochondrial respiration can occur in genes encoded by nuclear or mitochondrial DNA. In mitochondrial DNA, deletions and mutations in protein coding regions have been associated with multiple cancers, including breast and prostate cancer (Wallace 2005). Sporadic and familial mutations inactivating the nuclear-encoded subunits of the succinate dehydrogenase (SDH) complex trigger the development of pheochromocytoma and paraganglioma (Lehtonen *et al*. 2004). Similarly, loss-of-function mutations in fumarate hydratase (FH) can contribute to leiomyomas and renal cell carcinomas (Sudarshan *et al*. 2009). Mutations in the SDHB, SDHC, and SDHD subunits of SDH impair the TCA cycle, resulting in reduced charging of the electron transport chain, decreased ATP production, the generation of reactive oxygen species (ROS), and elevated succinate levels. Recently, mutations in Sdh5, a protein

that associates with the SDH complex and may mediate incorporation of FAD, have been identified in familial paraganglioma (Hao *et al*. 2009).

It stands to reason that impaired oxidative phosphorylation in the mitochondria would lead to compensatory increases in glycolysis, but it is critical to identify the mechanisms that mediate increased glycolysis under these circumstances. One possible explanation is that rising AMP levels in cells trigger allosteric activation of glycolytic enzymes, such as phosphofructokinase 1 (PFK1). However, the carcinogenic effect of an AMP-dependent activation of glycolysis is likely to be counteracted by the tumor suppressor effects of the AMP-activated protein kinase (AMPK). AMPK can phosphorylate and activate p53 tumor suppressor activity and directly inactivate the pro-growth kinase complex known as mammalian target of rapamycin complex 1 (mTORC1; Jones *et al*. 2005). Active p53 facilitates glucose oxidation via the TCA cycle by driving the production of cytochrome *c* oxidase assembly factor SCO2 (Matoba *et al*. 2006). Thus, a rise in AMP as a consequence of impaired TCA cycle function is not likely to contribute to oncogenic metabolism.

An alternative mechanism for activation of glycolysis in SDH mutant cells is based on the integration of key metabolites in the regulation of the hypoxia inducible factor-1α (HIF-1α). When HIF-1α pairs with the HIF-1β-binding partner, it forms a transcription factor that binds and activates the promoters of multiple glycolytic genes (Wang *et al*. 1995). In resting cells, proline hydroxylase enzymes (PHDs) hydroxylate two prolines in HIF-1α, targeting the protein for degradation by a Von Hippel-Lindau (VHL)-linked ubiquitin ligase complex (Bruick & McKnight 2001, Epstein *et al*. 2001, Ivan *et al*. 2002). PHD enzymes consume oxygen and α-ketoglutarate (also known as 2-oxoglutarate) and generate succinate in the process of hydroxylating proline residues in HIF-1α (Fig. 2). Decreased oxygen abundance is sufficient to inhibit HIF-1α proline hydroxylation, preventing HIF-1α degradation by the VHL ubiquitin ligase complex, and thereby triggering the accumulation of HIF-1α/HIF-1β transcriptional complexes on the promoters of glycolytic genes.

The exquisite sensitivity to oxygen abundance suggests that PHD enzymes may also be sensitive to the concentrations of the other metabolites involved in the reaction, namely α-ketoglutarate and succinate. An important advance was the demonstration that SDH-deficient cells accumulate increased succinate, impairing PHD activity and triggering HIF-1α accumulation (Selak *et al*. 2005). This observation was supported by

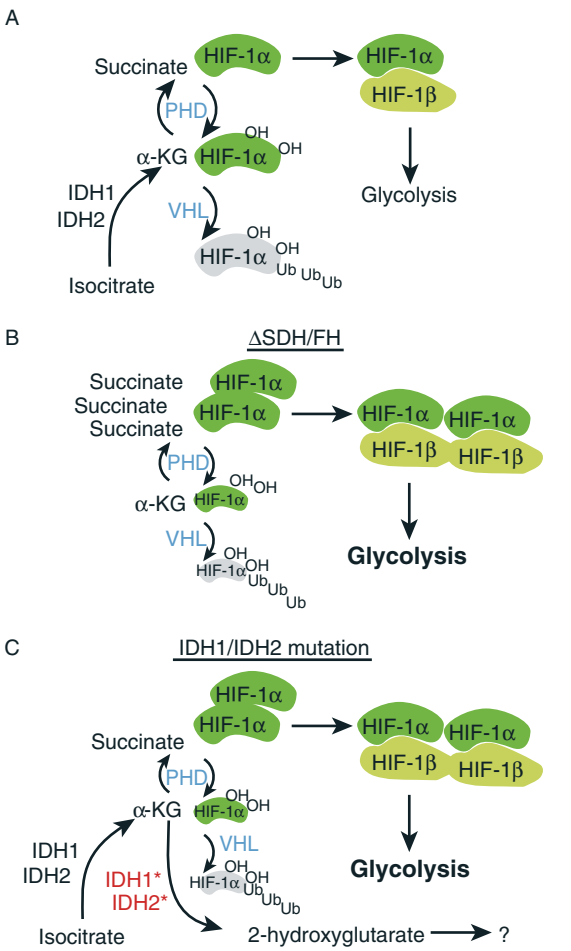

Figure 2 Regulation of HIF-1α in response to mutations in metabolic enzymes. (A) HIF-1α is hydroxylated at proline residues by the PHD enzymes, a reaction that requires α-ketoglutarate and produces succinate. Hydroxylated HIF-1α is ubiquitinated by a VHL-containing ubiquitin ligase. (B) Mutations in SDH or FH enzymes trigger accumulation of succinate, impairing HIF-1α hydroxylations. Increased HIF-1α drives increased glycolysis. (C) Mutant forms of IDH1 or IDH2 (marked with an asterisk) may reduce the availability of α-ketoglutarate, restricting HIF-1α hydroxylation and activating glycolysis. An alternative model proposes that mutated IDH enzymes acquire a novel activity, producing increased 2-hydroxyglutarate. The effects of 2-hydroxyglutarate on the signal transduction and metabolism of transformed cells are not yet well described.

measurement of increased succinate and HIF-1α levels in primary paraganglioma tumor samples (Pollard *et al*. 2005). Familial cancer-predisposing mutations in SDH and FH have established the principle that mitochondrial injury can be a primary event in oncogenesis, but the overall impact of mitochondrial function in the understanding of carcinogenesis has been diminished because of the relatively small proportion of cancers that are affected by these mutations.

With greater frequency than the SDH/FH mutations, mutations in isocitrate dehydrogenase 1 (IDH1) and the related enzyme IDH2 have been detected in glioblastoma and acute myeloid leukemia (AML; Parsons *et al.* 2008, Mardis *et al.* 2009). Genome sequencing of glioblastoma and AML has identified mutant forms of IDH1 or IDH2 in ~33% of cytogenetically normal leukemias (Marcucci *et al.* 2010). In one model, it is proposed that these mutations cause loss-of-function in the IDH1/IDH2 enzymes, decreasing the availability of α-ketoglutarate, and thus depriving the cells of a key substrate needed to carry out PHD-dependent HIF-1α hydroxylation (Fig. 2; Zhao *et al.* 2009). However, it has also been reported that glioblastomas and AML cells do not have decreased levels of α-ketoglutarate, and instead that IDH mutant enzymes have a gain-of-function in which they produce a new metabolic product: 2-hydroxyglutarate (Dang *et al.* 2009, Gross *et al.* 2010, Ward *et al.* 2010). Key experiments to determine the activity of PHD enzymes in IDH mutant tumors are required to determine whether the loss-of-function or gain-of-function mechanisms best explains the oncogenic effects of IDH mutations.

Biosynthetic contributions

Many of the biosynthetic pathways in the cell originate with the conversion of glycolytic intermediates into biosynthetic precursors. Although glycolysis generates a low yield of ATP relative to oxidative forms of metabolism, glycolysis can provide a rich source of biosynthetic intermediates that can be used for amino acid production, nucleic acid production, and glycerol synthesis. Several biosynthetic pathways have demonstrated critical roles in cancer.

A key biosynthetic pathway fed by the first steps of glycolysis is the pentose phosphate pathway (PPP). In the PPP, 6-phosphoglucose is diverted from the glycolytic pathway to generate pentose sugars and NADPH, an important reducing equivalent in the cell. Enhanced activation of the PPP has a number of pro-oncogenic effects. In xenograft models of breast cancer, tropism for brain metastasis was shown to correlate with increased expression of PPP enzymes and the production of NADPH (Chen *et al.* 2007*a*). Similarly, three-dimensional culture models of breast cancer oncogenesis have reported increased production of NADPH by the PPP as an important metabolic pathway for maintaining the survival of basement membrane-detached cells that accumulate in luminal structures (Schafer *et al.* 2009).

NADPH production benefits cancer cells via multiple mechanisms: facilitating biosynthetic reactions, detoxifying ROS, and initiating signal transduction that controls caspase 2. The key NADPH-dependent biosynthetic reactions are fatty acid synthesis, cholesterol synthesis, and nucleotide biosynthesis. In hormone-responsive tumors, fatty acid synthesis is increased by hormone receptor signaling, and inhibitors of fatty acid synthesis are known to be cytotoxic for cancer cells *in vivo* and *in vitro* (Kuhajda 2000, Guo *et al.* 2009). Increased fatty acid synthesis has been linked to poor prognosis in breast cancer and prostate cancer (Kuhajda *et al.* 1989, Shurbaji *et al.* 1996). It is thought that increased fatty acid synthesis is important for lipid modification of signal transduction subunits and the synthesis of new membrane in dividing cells (see below).

NADPH production is also critical for detoxifying ROS. Although increased ROS has been noted in many types of cancers (Toyokuni *et al.* 1995), it is also clear that transformed cells must balance ROS levels to maintain cell viability and optimal proliferative capacity. Indeed, overproduction of ROS on treatment with chemotherapeutics, such as 5-fluorouracil and doxorubicin, is thought to be an important mechanism for inducing cell death (Hwang *et al.* 2001). Conversely, reduction of ROS in cancer cells promotes apoptosis resistance via two mechanisms. In one mechanism, suppression of ROS promotes cell survival by increasing the capacity of cells to sustain metabolism through increased fatty acid oxidation (Schafer *et al.* 2009). Fatty acid oxidation can sustain cancer cell viability when other metabolic pathways such as glycolysis are unavailable (Buzzai *et al.* 2005). Furthermore, in some cell types the pro-apoptotic activity of cytochrome *c* can be regulated by the reducing potential of the cell (Vaughn & Deshmukh 2008). ROS-oxidized cytochrome *c* is more potent in inducing caspase 9 activation than the reduced form of cytochrome *c*, after mitochondrial permeabilization. Prevention of cytochrome *c* oxidation was shown to be dependent on the production of anti-oxidants by the PPP (Vaughn & Deshmukh 2008).

There is evidence that the PPP also contributes to direct regulation of pro-apoptotic proteins through NADPH-induced phosphorylation control of caspase 2. Caspase 2 is a potentially useful chemotherapeutic target because of its ability to mediate cell death via a mechanism that is independent of p53 and insensitive to overexpression of pro-survival Bcl-2 family proteins (Sidi *et al.* 2008). High levels of NADPH induce the activity of the calcium/calmodulin-dependent protein kinase II (CaM kinase II), which in turn phosphorylates caspase 2 at S315, preventing caspase 2 activation by providing a phospho-serine-binding site

for 14-3-3 family proteins (Nutt *et al*. 2005, 2009). However, there is a need for more extensive analysis of caspase 2 regulation in the context of transformed cancer cells before these mechanisms can be fully explored for chemotherapeutic targeting. Importantly, caspase 2 is also suggested to be a critical regulator of cell cycle checkpoints in response to irradiation, indicating that metabolic regulation of caspase 2 should also be considered for combination with therapeutics that disrupt cell cycle progression and DNA repair (Kitevska *et al*. 2009).

One mechanism controlling flux of glycolytic intermediates to the PPP versus glycolysis is regulated in part through the effects of p53 and its transcriptional target TP53-induced glycolysis and apoptosis regulator (TIGAR). The TIGAR protein contains a domain that is homologous to the phosphatase domain of 6-phosphofructo-2-kinase/fructose-2,6 bisphosphatase (PFK2). Increased expression of TIGAR suppresses the flux of glucose to downstream elements of the glycolytic pathway, thereby increasing the abundance of glucose-6-phosphate, which then enters the PPP (Bensaad *et al*. 2006). Increased TIGAR correlated with protection from apoptosis induced by doxorubicin (adriamycin) and other apoptotic stimuli associated with the generation of ROS (Bensaad *et al*. 2006). Interestingly, TIGAR can also regulate ROS levels in cells through effects on autophagy, a process that allows cells to consume intracellular macromolecules under conditions of nutrient deprivation (Bensaad *et al*. 2009). The ability of TIGAR to regulate the switch between bioenergetic production through glycolysis and biosynthetic production suggests that the expression of this molecule in various cancer types may be key for determining the optimal metabolic pathways to be targeted in chemotherapy.

Beyond the regulation of PPP versus glycolysis in the first few steps of glycolysis, the utilization of glucose-derived carbons for glycolysis or biosynthesis is also affected by the differential expression of pyruvate kinase (PK) isoforms. PK catalyzes the penultimate step of glycolysis, converting phosphoenolpyruvate to pyruvate and generating ATP as a result. Alternative mRNA splicing triggers the production of two isoforms of PK in most tissues: PKM1 and PKM2. Compared to PKM2, PKM1 can form a tetrameric structure that has a higher affinity for its substrate (phosphoenolpyruvate) and is associated with increased cellular ATP:ADP ratios (Mazurek *et al*. 2005). PKM2 forms a dimeric structure, with decreased affinity for phosphoenolpyruvate and can be allosterically regulated by binding to tyrosine phosphorylated peptides and by 2,6-bisphosphofructose (Christofk *et al*. 2008*b*). These characteristics might suggest that PKM1 would be associated with oncogenesis. However, PKM2 expression is tightly associated with cell transformation.

By preferentially expressing PKM2 instead of PKM1, cancer cells sacrifice the potential to generate ATP to increase the abundance of upstream metabolites in the glycolytic pathway. As described above, the diversion of glucose 6-phosphate into the PPP can promote the synthesis of fatty acids and cholesterol through the production of NADPH. However, decreased PK activity also increases the abundance of glyceraldehyde-3-phosphate and 3-phosphoglycerate, which can contribute to the synthesis of nucleic acids and amino acids respectively. Thus, the expression of PKM2 suggests that a major destination of carbon derived from glucose in cancer cells is biosynthesis of macromolecular structures.

The importance of PKM2 expression during cellular transformation is demonstrated by substituting PKM1 expression for PKM2 in cancer cell lines (Christofk *et al*. 2008*a*). *In vitro*, there is no difference in the growth rate of cancer cells with PKM1 expression. However, xenograft experiments reveal a striking decline in the growth rate of PKM1-expressing tumor cells. This result indicates that *in vivo* conditions require greater coordination of metabolic pathways to support biosynthesis, and that PKM2 expression is a critical enzyme that supports this process.

Expression of PKM1 versus PKM2 can differ by tissue type, but PKM2 expression is correlated with cell cycle progression and cellular transformation (Christofk *et al*. 2008*a*, Clower *et al*. 2010). The splicing factors known as polypyrimidine tract-binding protein (PTB – also known as PTBP1 or heterogeneous nuclear ribonucleoprotein A1 (hnRNP1)), hnRNPA1 and hnRNPA2 mediate the switch from expression of PKM1 to PKM2 (Clower *et al*. 2010, David *et al*. 2010). Increased abundance of PTB, hnRNP1, and hnRNP2 is increased on expression of the pro-glycolytic transcription factor c-Myc (David *et al*. 2010). Increased c-Myc correlated with PKM2 expression in gliomas, indicating that c-Myc activity may be important for reprograming cellular metabolism to support oncogenesis in this tumor type.

PTB and hnRNP proteins regulate PKM splicing by binding to sites proximal to PKM1-specific Exon 9, inhibiting Exon 9 splicing and resulting in splicing of the PKM2-specific Exon 10. Knockdown experiments reveal that hnRNPA1 and hnRNPA2 function redundantly to inhibit PKM1 production, while PTB knockdown alone was sufficient to restore expression of PKM1 (Clower *et al*. 2010, David *et al*. 2010). Unexpectedly, increased PKM1 expression on

knockdown of PTB or hnRNPs suppressed lactate production, despite the increased catalytic activity of PKM1 to produce pyruvate, relative to PKM2 (Clower *et al*. 2010). This may suggest that the PKM1/PKM2 paralogs affect the destination of glucose-derived carbon (TCA cycle versus lactate), or that the PTB and hnRNP proteins affect multiple enzymes in the glycolytic pathway.

Increased PTB expression has been noted in some cancers, although expression levels do not in all cases correlate with metastatic potential or stage of malignancy (Jin *et al*. 2000, He *et al*. 2004, Wang *et al*. 2008). Interestingly, knockdown of PTB in cancer cell lines suppresses cell proliferation in semisolid media. Owing to the likelihood that alterations in splicing factors affect multiple aspects of cell function, it will be necessary to investigate whether the primary effects of suppressing PTB are mediated by alterations in PKM isoforms or whether other pathways are also critical.

Temporal programing

Although it is clear that regulation of metabolic pathways can solve bioenergetic and biosynthetic problems for cancer cells, a new perspective on the functional benefits of metabolic pathways has emerged from studies of the temporal regulation of metabolism. Beginning in yeast grown in continuous concentrations of nutrients, Tu *et al*. (2005) observed oscillations in the oxygen consumption of yeast, which correlated with entry of cells into S phase of the cell cycle. In the G_1 phase, oxidative metabolism is engaged, while ribosome and protein synthesis generate intense demand for ATP. During cell replication, cells switch to glycolytic metabolism, thereby reducing the risk of genomic damage due to ROS generated by oxidative metabolism (Chen *et al*. 2007*b*). These observations establish the concept that cells coordinate their metabolic program in synchrony with major cellular functions, such as cell cycle progression or ribosome biogenesis.

The importance of temporal coordination in setting metabolic programs in cancer cells is suggested by the observation of decreased expression of the *period* (*PER1*) gene in human samples of non-small cell lung carcinoma and breast cancer (Gery *et al*. 2006, 2007*a*). The *period* genes (*Per1*, *Per2*, and *Per3*) are expressed in most mammalian tissues to regulate the metabolic machinery in anticipation of events such as nutrient influx or increased energetic demand. Period proteins associate with cryptochrome (CRY) proteins to regulate the activity of the central circadian rhythm transcription factors BMAL1 and Clock. Circadian rhythm is established through a negative feedback loop in which period:cryptochrome complexes inhibit BMAL1:Clock, until the period:cryptochrome complexes are dissociated or degraded. The subsequent increases in BMAL1:Clock transcriptional activity drive expression of period and cryptochrome, which accumulate until BMAL1:Clock activity is restrained (Fig. 3). The BMAL1:Clock transcription factor regulates the expression of enzyme components of multiple metabolic pathways, including glycolysis. Please see Takahashi *et al*. (2008) for a recent review.

The expression levels of period proteins cycle in response to changes in the upstream central circadian rhythm genes *BMAL1* and *Clock*, and also to various inputs ranging from metabolism to signal transduction (Sahar & Sassone-Corsi 2009). Transcriptional targets controlled by the circadian clock include both cell cycle regulators and key metabolic enzymes such as PK, suggesting that the coordination of the replicative cell cycle and the metabolic cycle could be mediated by the *period* genes (Lamia *et al*. 2008). Inactivation of the related gene *Per2* in mice triggers hyperplasia of the salivary glands, epidermal teratomas, and lymphoma. (Fu *et al*. 2002). In breast cancer, deregulated expression of Per2 has been tied to estrogen receptor activation (Gery *et al*. 2007*b*). A modest increase in breast cancer incidence has been noted in women whose circadian rhythms are altered due to nighttime working hours (Schernhammer *et al*. 2006).

Recently, AMPK, a major effector of the tumor suppressor activity of LKB1, was shown to regulate circadian rhythms by phosphorylating and destabilizing the circadian transcription factor CRY1 (Lamia *et al*. 2009). AMPK suppresses oncogenic signal transduction by opposing the activation of mTORC1,

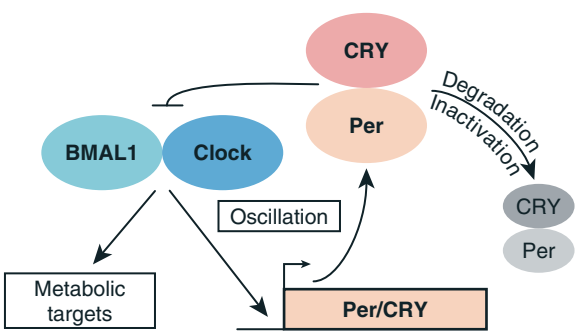

Figure 3 The circadian clock in cancer. BMAL1:Clock proteins regulate transcription of their inhibitors, *period*, and *cryptochrome* genes. Degradation of period and cryptochrome proteins activates BMAL1:Clock in the regulation of a large number of transcriptional targets, including genes that regulate glycolysis and other metabolic pathways.

a critical kinase that controls cell growth, survival, and metabolism. Cry1$^{-/-}$ mice have reduced tumorigenesis in a p53$^{-/-}$ background, suggesting that an additional mechanism for AMPK tumor suppressor activity could be mediated by phosphorylation and destabilization of CRY1 (Ozturk *et al*. 2009). Further study will be needed to determine whether the seemingly contradictory effects in oncogenesis of *CRY1*$^{-/-}$ (decreased cancer) and *Per2*$^{-/-}$ (cancer susceptibility) are reflected in opposing effects on cellular metabolism.

If temporal programing is a key determinant of the activation of glycolysis, then an important question is whether cancer therapy can be timed to maximize chemotherapeutic response by anticipating alterations in metabolic pathways. Although the mechanisms are not fully elucidated, there are a number of reports of increased chemotherapeutic responses when administration of drugs is timed according to diurnal cycle (Haus *et al*. 1972, Boughattas *et al*. 1989, Takahashi *et al*. 2008). In mice, B-cell cytotoxicity in response to cyclophosphamide varies according to the circadian clock, an effect that can be genetically replicated by increased cytotoxicity in *Clock* mutant mice (Gorbacheva *et al*. 2005). Conversely, *CRY1*-deficient B cells (which have increased BMAL1:Clock activity) are resistant to the cytotoxic effects of cyclophosphamide. Importantly, the differential responses of *Clock*- or *CRY1*-deficient mice were not linked to alterations in cyclophosphamide pharmacokinetics. Considering these results in the context of increased lymphoma in *Per2*$^{-/-}$ mice, it will be interesting to learn in future studies whether the effects of circadian regulation are related to cell-intrinsic effects on metabolism and apoptosis.

What do we learn from the various roles of glycolysis in carcinogenesis? If increased glucose metabolism is a product of bioenergetic demand, then we must target the metabolites and signaling proteins, such as HIF-1α, which are critical to cancer cell adaptation to energetic stress. Activation of the protein kinases Akt and mTORC1 is strongly linked to increased glycolytic metabolism, as described in a review (Robey & Hay 2009). A recently described mechanism that may contribute to Akt regulation of glycolysis is the regulation of mTORC1 signaling pathway by the forkhead box subclass O (FOXO) transcription factors (Chen *et al*. 2010, Khatri *et al*. 2010). In this model, oncogene-induced Akt would inactivate the FOXO transcription factors, activating mTORC1-stimualted glycolysis due to decreased function of the tuberous sclerosis complex 1 (TSC1)–TSC2 tumor suppressor complex.

In the scenario where glycolytic metabolism contributes to biosynthetic pathways, a different set of chemotherapeutic targets emerges – those metabolic modifiers that reroute glucose-derived carbon toward biosynthetic pathways, such as TIGAR or PKM2. Association of glycolysis with cell cycle progression, perhaps coordinated by circadian clock proteins, indicates a third avenue to regulate glycolytic metabolism. Although increased glucose metabolism is commonplace in cancer, we predict that glycolysis is enlisted for different purposes in distinct tumors. Effective targeting of the glycolytic program in cancer will therefore require investigation of the contributions of glucose metabolism across the spectrum of cancer.

Glutaminolysis: the 'other' Warburg effect?

> 'In conclusion we may mention a remarkable connection, the significance of which is not yet clear, which exists between the glycolytic action of the cell and its action on nitrogen-containing substances. If sections of Flexner rat-carcinoma are placed in sugar-free Ringer's solution at body-temperature, considerable quantities of ammonia develop.' (Warburg *et al*. 1931).

If glucose metabolism can be successfully targeted in cancer, will limiting glycolysis be sufficient to eliminate the bioenergetic and biosynthetic underpinnings for transformed cell growth? Unfortunately, some oncogenic events can activate metabolic pathways that can substitute for some or all of the effects of glucose metabolism. One example is the metabolic program induced by oncogenic expression of the Myc transcription factor. *Myc* is a potent oncogene that is well known for its ability to drive cell cycle progression coupled with increased glycolytic metabolism (Dang *et al*. 2006). Increased glucose metabolism driven by Myc might suggest glucose-dependent cancer cell growth and survival, similar to previous observations of glucose-dependent cell survival driven by Akt (Gottlob *et al*. 2001, Plas *et al*. 2001). However, Myc-expressing cells are hypersensitive to limited oxygen availability, rather than glucose metabolism (Brunelle *et al*. 2004, Yuneva *et al*. 2007). Dependence on oxidative metabolism in Myc-transformed cells may be due to increased glutamine metabolism (Yuneva *et al*. 2007).

Myc has been shown to directly regulate the transcription of glutamine transporters (Wise *et al*. 2008),

but indirect effects on glutaminolysis have also been described. Myc promotes increased expression of glutaminase, an enzyme that converts glutamine to glutamate, by repressing the expression of miRNA-23a/b (Gao *et al*. 2009). In addition, Myc enhances the overall capacity of the cell to conduct oxidative metabolism by activating the transcription of genes required for increased mitochondrial biogenesis (Li *et al*. 2005).

Increased expression of enzymes mediating increased glutamine metabolism has been correlated with cell transformation (Knox *et al*. 1969). Glutamine contributes to biosynthesis by direct incorporation into nascent polypeptides, by supporting NADPH pools, or as a precursor for *de novo* pyrimidine synthesis (Fig. 4). Glutamine also contributes to cellular bioenergetics by entering the TCA cycle after conversion first to glutamate then to α-ketoglutarate. However, decreases in ATP and NAD:NADH ratios were not detected in glutamine-deprived cells (Yuneva *et al*. 2007). Global metabolic analysis of glutamine-consuming glioblastoma cells suggested that glutamine metabolism provided significant contributions to cellular levels of NADPH and glutamine-derived carbon contributed to phospholipid synthesis (DeBerardinis *et al*. 2007, Wise *et al*. 2008). By-products of glutamine consumption are excess quantities of lactate, alanine, and ammonia – the latter reminiscent of Warburg's observation of ammonia production in carcinoma samples.

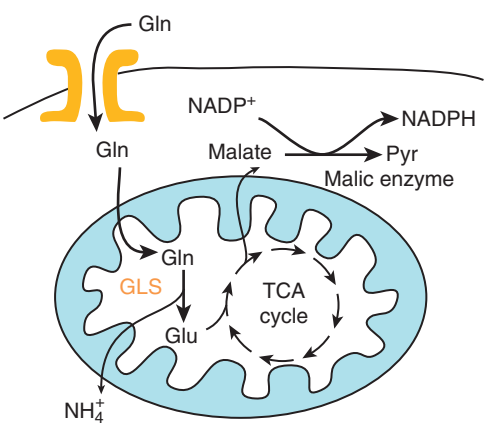

Figure 4 Glutamine metabolism in transformed cells. c-Myc induces the expression of glutamine transporters at the plasma membrane and acts indirectly through miR23 to increase the expression of glutaminase (GLS). Glutamine contributes to several biosynthetic pathways, including the production of NADPH through malic enzyme catalysis of malate exported from the mitochondria.

Combining glutaminolysis and glycolysis

Similar to the Akt pathway, Myc has the ability to activate glycolysis. However, Myc does not sensitize cells to glucose-limiting conditions, while cells with activated Akt are hypersensitive to interruptions in glycolysis. Increased glutaminolysis can explain the resistance of Myc-expressing cells to glucose-limiting conditions, but it is interesting to consider the reverse situation: why do Akt-expressing cells fail to acquire another sustaining bioenergetic program, such as glutaminolysis or autophagy (Lum *et al*. 2005), under glucose-limiting conditions? Direct comparison of the metabolic pathways induced by Myc and Akt provides a possible explanation: Akt cells are less prepared for the switch to oxidative forms of metabolism due to insufficient mitochondrial biogenesis (Fan *et al*. 2010).

The unveiling of Myc-induced glutaminolysis in conjunction with glucose metabolism provides a cautionary note for the targeting of glucose metabolism in cancer therapy. The studies of Myc-induced metabolism demonstrate that increased glucose metabolism does not necessarily render cells hypersensitive to interruptions in glucose metabolism. Although we and others have suggested otherwise in the past (Plas & Thomas 2009), the observation of increased glucose metabolism by fluorodeoxyglucose positron emission tomography (FDG-PET) or other approaches is not sufficient to suggest chemotherapeutic targeting of glycolytic metabolism. It is necessary to understand and target the complete metabolic effects of oncogenic mutations. The power of this approach was recently demonstrated in glioblastoma cells with activated metabolism of both glucose and glutamine – cell survival was only compromised on inhibition of both glycolysis and glutaminolysis (Yang *et al*. 2009).

Fatty acid metabolism: metabolism for long-lived cells

Similar to glucose and glutamine, fatty acid metabolism supports both the biosynthetic and the bioenergetic requirements for cell proliferation and survival. Fatty acids can be converted to triglycerides for storage, phospholipids for membrane biogenesis or oxidized to CO_2 for energy production. In proliferating cells, fatty acids are channeled for lipid synthesis, which contributes to membrane production and posttranslational lipid modification of proteins (Bauer *et al*. 2005). In addition, fatty acid consumption through β-oxidation can provide a key alternative pathway that can support cancer cell survival when glucose metabolism becomes limiting. The ability to

efficiently support either biosynthetic or bioenergetic needs may explain an apparent connection between fatty acid metabolism in long-lived cell types, such as memory lymphocytes and cardiac myocytes (Pearce *et al*. 2009, Chung *et al*. 2010).

Fatty acid synthesis

Oncogenic mutations alter the expression and activity of two key enzymes involved in fatty acid synthesis, fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC; Yoon *et al*. 2007). Under conditions where glycolysis or glutaminolysis produces excess pyruvate, increased expression of FASN and ACC promotes *de novo* fatty acid synthesis to maintain the supply of lipids needed for membrane production and protein modifications (Fig. 5). Tumors preferentially use the newly synthesized fatty acids for membrane production rather than utilize dietary fatty acids (Medes *et al*. 1953, Ookhtens *et al*. 1984). ACC produces malonyl-CoA, a key intermediate in fatty acid synthesis that also serves as an allosteric inhibitor of fatty acid oxidation. In some cells, if ACC production of malonyl-CoA drops, fatty acids can be diverted to generate bioenergetic equivalents through mitochondrial β-oxidation. ACC- and FASN-mediated synthesis of palmitate in breast cancer cells is required to maintain viability (Chajes *et al*. 2006). Multiple inhibitors of FASN have been developed and show promising effects *in vitro* and in xenografts (Menendez & Lupu 2007).

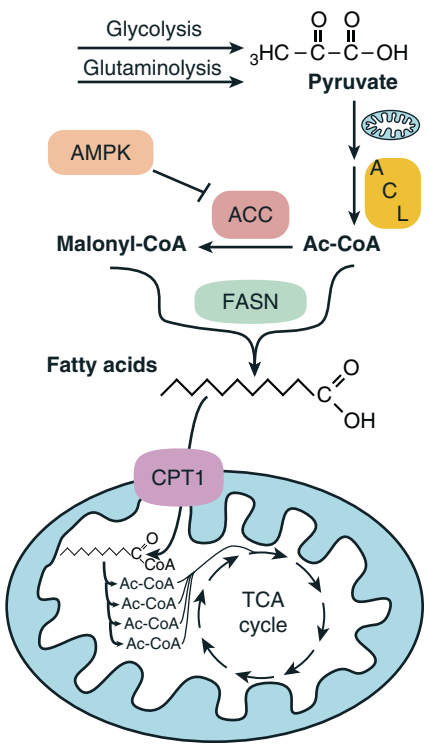

Figure 5 Fatty acid metabolism in transformed cells. Pyruvate derived from upstream metabolic pathways is converted to citrate in the mitochondria then exported to the cytosol, where it fuels ATP citrate lyase (ACL) production of acetyl-CoA. The acetyl-CoA carboxylase (ACC) enzyme produces malonyl-CoA from acetyl-CoA, which together is used in *de novo* fatty acid synthesis by the fatty acid synthase (FASN) enzyme. Fatty acids can also be catabolized by β-oxidation. Key steps in this process are the entry of fatty acids into the mitochondria via the CPT1 transporter and inhibition of ACC activity by AMPK. See text for details.

Increased expression of FASN and related enzymes critical for fatty acid synthesis has been noted in multiple types of cancer, including breast, prostate, thyroid, and ovarian cancers (Menendez & Lupu 2007). FASN expression can be induced by oncogenic activation of the phosphatidylinositol 3-kinase (PI3K) pathway or by the mitogen-activated protein kinase (MAPK) pathway (Van de Sande *et al*. 2002, Yang *et al*. 2002). The effects of the PI3K and MAPK pathways are proposed to be mediated through control of the sterol regulatory element-binding protein-1 (SREBP-1) transcription factors, master regulators of fatty acid synthesis (Eberle *et al*. 2004).

SREBP-1a and SREBP-1c are protein products of the *Srebf1* gene, differing in their amino termini due to alternative promoter usage. Downstream of Akt, induction of SREBP-1 proteins has been reported to be rapamycin sensitive (Yoon *et al*. 2007) in HER2$^+$ breast cancer cells, but rapamycin insensitive in glioblastoma cells (Guo *et al*. 2009). Interestingly, cells expressing elevated SREBP-1 are sensitive to the induction of apoptosis in response to FASN inhibitors *in vitro* and in xenograft models (Guo *et al*. 2009). It is not clear why acute inhibition of ongoing fatty acid synthesis induces apoptosis instead of a cytostatic cell cycle arrest. Although activation of SREBP-1 is associated with increased signal transduction, SREBP-1 is also regulated through proteolytic cleavage and by the ubiquitin-proteasome system (Eberle *et al*. 2004). Thus, significant mechanisms for oncogene regulation of this important transcription factor remain to be investigated.

FASN requires a source for acetyl CoA to mediate increased fatty acid synthesis. In cells with oncogenic activation of PI3K/Akt, acetyl CoA is generated by activation of ATP citrate lyase (ACL; Bauer *et al*. 2005, Hatzivassiliou *et al*. 2005). Acting on excess citrate generated under nutrient-rich conditions, ACL converts citrate to acetyl CoA and oxaloacetate. Akt directly phosphorylates and activates ACL catalytic activity (Berwick *et al*. 2002). Similar to FASN, ACL has emerged as a promising target for disrupting

oncogene-induced metabolism. ACL knockdown or treatment with pharmacologic inhibitors induced a cytostatic block of cancer cell growth *in vitro* and in a xenograft model (Hatzivassiliou *et al*. 2005, Migita *et al*. 2008).

Fatty acid oxidation

Cancer cells can shift to oxidation of nonglucose carbon to maintain mitochondrial membrane function and support cell survival. For example, stromal cell interactions can promote a metabolic shift to the oxidation of fatty acids versus glucose to support leukemia (Samudio *et al*. 2008). Fatty acid oxidation can rescue matrix detachment and promote anchorage-independent survival in an *in vitro* model of breast cancer (Schafer *et al*. 2009). Indeed, activating fatty acid oxidation in Akt-transformed cells protects from apoptosis in the absence of glucose (Buzzai *et al*. 2005).

Fatty acid β-oxidation is controlled by the carnitine palmitoyl transferase (CPT) system and the availability of cofactors NAD^+ and FAD^+ (Eaton *et al*. 1996). The CPT transport system facilitates the entry of fatty acids into the mitochondria (Fig. 5). CPT-1 is the first and rate-limiting step of fatty acid transport. CPT-1 import of fatty acids into the mitochondria is inhibited by malonyl-CoA, the product of the ACC fatty acid synthesis enzyme, as described above. Activation of AMPK promotes import of fatty acids by phosphorylating and inhibiting ACC, relieving malonyl-CoA repression of CPT-1 (Davies *et al*. 1990).

In addition to allosteric regulation by malonyl-CoA, CPT-1 fatty acid transport activity is also controlled through transcriptional regulation by upstream signaling pathways. In particular, both growth factors and oncogenic mutations can repress *CPT1A* transcription by activating the PI3K/Akt pathway (DeBerardinis *et al*. 2006). Interestingly, re-activation of the dormant fatty acid oxidation pathway via AMPK activation can provide Akt-transformed cells with an alternative energy source that can substitute for glycolysis (Buzzai *et al*. 2005).

Aggressive cancer cell lines accumulate high levels of free fatty acids, at least partly as a result of increased expression of monoacylglycerol lipase (MAGL; Nomura *et al*. 2010). Accumulation of fatty acid end products, such as palmitate, contributes to the generation of ROS and ceramide, a proapoptotic second messenger (Obeid *et al*. 1993). Therefore, cancer cells with increased fatty acid levels require a mechanism to balance lipid synthesis and cytotoxicity. There are two mechanisms that can satisfy this balance of increased fatty acid synthesis, conversion to triglycerides for storage or β-oxidation for energy production. Upregulation of CPT-1 mRNA in pancreatic β cells prevents death induced upon palmitate accumulation (Wan *et al*. 2010). Inhibition of fatty acid oxidation in human leukemia cells through the loss of CPT-1 by shRNA or treatment with etomoxir sensitized cells to chemotherapy (Samudio *et al*. 2010). Additionally, MAGL inhibitors suppress the carcinogenic potential of aggressive cancer cells (Nomura *et al*. 2010).

Of the three metabolic programs that we have considered in this study, fatty acid metabolism holds a number of key advantages for chemotherapeutic targeting. Specificity of FASN inhibitors for cancer cells has been tested extensively with promising results, and the fatty acid oxidation inhibitor etomoxir has been tried in short-term protocols for cardiac disease (Schmidt-Schweda & Holubarsch 2000, Holubarsch *et al*. 2007, Menendez & Lupu 2007). Xenograft tests of efficacy have yielded supportive results. Furthermore, both glycolysis and glutaminolysis appear to mediate their oncogenic effects by contributing to NADPH production and fatty acid synthesis. It is now time to experimentally define the genetic and cell growth parameters that can maximize cytotoxicity elicited by fatty acid metabolism inhibitors, and translate this approach for cancer chemotherapy.

Signaling pathway control of metabolism

Specific mutations in oncogenes and tumor suppressors are associated with distinct metabolic programs in cancer. Highlighted below are oncogenic mutations that activate metabolic programs in cancer through alterations in signaling pathways.

Akt-induced glycolysis is a common feature in cancer due to the prevalence of activating mutations in PI3K or inactivating mutations in the phosphatase and tensin homolog (PTEN). Both of these mutations increase the abundance of 3,4,5-phosphatidylinositol trisphosphate (PIP_3), a critical upstream activator of Akt. The requirement for PIP_3 in Akt activation can be alleviated by mutations within Akt itself (Carpten *et al*. 2007). Finally, numerous activating events in upstream tyrosine kinases, such as BCR–ABL, induce Akt signaling and glycolysis by activating PI3K (Boren *et al*. 2001, Gottschalk *et al*. 2004). Activated growth factor receptors can also trigger increased fatty acid synthesis through SREBP-1-mediated transcriptional control of FASN (Swinnen *et al*. 2000, Kumar-Sinha *et al*. 2003). Owing to its central role in regulating the activation of Akt and glycolysis, PI3K is a major target

for chemotherapeutic drug development, with several compounds now in clinical trial (Liu *et al*. 2009).

Downstream of Akt, glycolysis is controlled by evolutionarily conserved substrates: the TSC2 tumor suppressor protein and the FOXO transcription factors (Brugarolas *et al*. 2003, Khatri *et al*. 2010). FOXO transcription factors regulate the production of TSC1, a chaperone protein that stabilizes TSC2 (Khatri *et al*. 2010). TSC2 is a negative regulator of the small GTPase protein known as Rheb, which in turn is critical for activating the protein kinase mTORC1. mTORC1 is a protein kinase complex consisting of the mTOR catalytic subunit in association with Raptor, LST8, and other cofactors. Activated Akt phosphorylates and inhibits the tumor suppressor-like functions of FOXOs and TSC2, activating Rheb and mTORC1. Loss of either FOXO transcription factors, TSC1 or TSC2, is sufficient to activate glycolysis (Khatri *et al*. 2010). Cancer-associated mutations have been reported in TSC1 and mTORC1 (Knowles *et al*. 2003, Sato *et al*. 2010).

Although PI3K/Akt signaling is sufficient to activate glycolysis, tumors can also activate glycolysis via Akt-independent mechanisms. Overexpresion of c-Myc through chromosomal translocation or gene locus amplification leads to direct transcriptional induction of glycolytic enzymes (Osthus *et al*. 2000). Transcriptional activation of glycolysis through the HIF-1α transcription factor is also triggered by mutations in the metabolic enzymes SDH and FH (Lehtonen *et al*. 2004, Sudarshan *et al*. 2009). p53 inactivation, one of the most common oncogenic mutations, can lead to increased glycolysis by reducing transcription of TIGAR and SCO2, which suppress glycolysis (Bensaad *et al*. 2006, Matoba *et al*. 2006).

Glutamine catabolism is emerging as an important contributor of NADPH required for nucleotide biosynthesis and carbon for the synthesis of lipids, proteins, and amino acids (DeBerardinis *et al*. 2007, Wise *et al*. 2008). The *c-myc* oncogene drives glutamine catabolism in part through suppression of miR-23a/b enhancing glutaminase expression rendering the cells dependent on glutamine to sustain viability (Gao *et al*. 2009).

Metabolic programs in chemotherapy

We have focused on the metabolic programs that are frequently activated in cancer, and how these programs contribute to oncogenesis. As our understanding of the role of metabolism in supporting oncogenesis grows, the key question will be how to exploit cancer cell metabolism to overcome apoptosis resistance and enhance chemotherapeutic responses. Recent approaches aim to interrupt the signaling pathways that lead to oncogenic changes in metabolism, or to directly inhibit the metabolic programs that cancer cells have acquired.

Targeting metabolism

Direct inhibition of glycolysis has long been considered as a chemotherapeutic strategy in cancer. We previously showed that apoptosis resistance mediated by Akt can be overcome by reducing cellular glycolysis (Plas *et al*. 2001). A number of compounds directly target the glycolytic pathway, including dichloroacetate (DCA), 2-deoxy-glucose (2-DG), and 3-bromopyruvate (3-Br). DCA is an inhibitor of pyruvate dehydrogenase kinase-1 (PDK-1), a kinase that regulates the flux of pyruvate into mitochondria. By inhibiting PDK-1, DCA redirects pyruvate away from the production of lactate, inducing pyruvate entry into the mitochondrial TCA cycle. DCA can induce increased mitochondrial function and apoptosis in primary glioblastoma cells, and showed therapeutic benefits in a small clinical trial (Bonnet *et al*. 2007, Michelakis *et al*. 2010). 2-DG competes with glucose as a substrate for hexokinase preventing glucose phosphorylation and oxidation via glycolysis. In animal models, 2-DG selectively induced cell death in transformed cells when used in combination with other anticancer therapeutics such as paclitaxel or histone deacetylase (HDAC) inhibitors (Maschek *et al*. 2004, Egler *et al*. 2008). Similarly, the hexokinase inhibitor 3-Br suppressed xenograft growth of human colorectal carcinoma cells containing activated alleles of Ras or Raf, though it was not clear whether this activity is due to cytotoxic versus cytostatic effects (Yun *et al*. 2009). Although glycolytic inhibitors demonstrate therapeutic potential in animal models, their application in the clinic is limited at best. A concern regarding glycolysis inhibition is raised by the observation of a selective advantage of colon cancer cells with mutations in K-Ras or B-Raf when glycolysis is inhibited (Yun *et al*. 2009). This suggests that glycolysis inhibition should be used in combination with other approaches that can prevent the emergence of therapy-resistant cancer cells.

Although direct inhibitors of fatty acid metabolism exhibit specific cytotoxicity for cancer cells *in vitro*, toxicity and bioavailability issues *in vivo* have prevented the translation of this approach into the clinic. One of the most attractive targets for inhibition in cancer chemotherapy is FASN, due to the high degree of FASN overexpression in cancer cells.

A single FASN polypeptide chain contains multiple enzymatic activities, and there is a range of inhibitors that target these enzymatic activities (Menendez & Lupu 2007). These inhibitors can induce cytotoxic effects in breast cancer cells, especially in combination with other chemotherapeutic agents including paclitaxel (Menendez *et al*. 2005). Etomoxir, an inhibitor of fatty acid oxidation, can sensitize leukemia and colon cancer cells to apoptosis (Hernlund *et al*. 2008, Samudio *et al*. 2010).

Targeting signal transduction

Therapeutics that target the Akt pathway are also being evaluated in the clinic with mixed results. The most widely used inhibitor is rapamycin, a compound that forms a complex with the cellular protein FKBP12 and inhibits the phosphorylation of substrates by mTORC1 (Abraham & Gibbons 2007). Rapamycin derivatives were approved by the FDA for various settings in renal cell carcinoma, based on an ability to extend median survival (Hudes *et al*. 2007, Motzer *et al*. 2008). Despite the successful application of these drugs, multiple studies have reported cytostatic but not cytotoxic effects of rapamycin and its analogues, resulting in disease stabilization but not regression (Bissler *et al*. 2008, Motzer *et al*. 2008, Wolpin *et al*. 2009). The lack of cytotoxicity may be related to a side effect of inhibition of mTORC1 – activation of Akt. Under homeostatic conditions, Akt activation is restrained by an mTORC1-regulated negative feedback loop. Treatment with rapamycin inactivates this restraining mechanism, resulting in increased Akt phosphorylation of multiple substrates (O'Reilly *et al*. 2006). It is possible that increased Akt signaling confers increased apoptosis resistance, a counterproductive effect in cancer chemotherapy. Dual mTOR/PI3K inhibitors, such as BEZ235, or mTORC1/mTORC2 inhibitors, such as WYE-125132, do not suffer from secondary activation of Akt, and consequently it is possible that these inhibitors will trigger cancer-specific cytotoxicity as opposed to cytostatic effects (Brachmann *et al*. 2009, Yu *et al*. 2010).

The antidiabetic compound metformin has significant potential for use in both cancer chemotherapy and cancer prevention, associated with its ability to impede mTORC1 signaling. Cancer cells respond to metformin by activating AMPK (Zhou *et al*. 2001). We have already described the ability of AMPK to phosphorylate important metabolic regulatory proteins, including p53, CRY1, and ACC. In addition, AMPK impedes mTORC1 signaling through stimulatory phosphorylation of TSC2, and inhibitory phosphorylation of raptor (Inoki *et al*. 2003, Gwinn *et al*. 2008). By impairing mTORC1 signaling, AMPK opposes the pathway that signals activation of glycolysis in cancers with upstream activating mutations. AMPK activation in response to metformin may also impair fatty acid synthesis in cancer cells through its effects on ACC and its ability to suppress the expression of SREBP-1 (Zhou *et al*. 2001). Metformin has been shown to delay or prevent carcinogenesis in diabetic patients (Evans *et al*. 2005). Incorporation of metformin into chemotherapeutic regimens is under development in multiple clinical trials. It will be important to determine whether metformin efficacy correlates with suppression of glycolysis, fatty acid synthesis, or other alterations in metabolism.

In the above examples, metabolic interventions induced cytotoxic effects, while signaling inhibitors were associated with cytostatic effects. Increasing the specificity of the anti-metabolites or improved targeting of signaling pathways is necessary to achieve cytotoxic responses that can be used in the clinic. Targeting multiple signaling nodes, as exemplified by BEZ235 inactivation of PI3K and mTOR, represents one strategy for improving cytotoxic responses. Combining anti-metabolites with pathway-specific therapeutics may also improve chemotherapeutic responses. Maximal cytotoxic responses may be achieved by disrupting the delicate balance between biosynthetic and bioenergetic metabolic pathways in cancer cells.

Conclusions

Cancer cells derive biosynthetic and bioenergetic benefits from oncogenic mutations that activate glycolysis, glutaminolysis, and fatty acid metabolism. The mechanisms that activate metabolism include signal transduction activation of metabolic genes, induction of metabolism in response to bioenergetic demand, and disruption of temporal control of metabolism. Counteracting metabolic alterations in cancer cells in chemotherapy may be achieved, but potential drawbacks with leading approaches, such as rapamycin or direct glycolysis inhibitors, have been described. This suggests that the differences in the 'metabolic programs' controlled by each oncogene will determine the potential efficacy of targeting metabolism in chemotherapy. As genomic sequencing of cancers finally reveals the full complement of oncogenic mutations driving a particular tumor, we must understand these mutations in terms of the metabolic programs that they control. In this way, metabolic targeting can be used in cooperation with other forms of targeted chemotherapy to improve cancer treatment outcomes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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