Cancer Cell
Special Collection
10 YEARS OF EXCITING CANCER RESEARCH SINCE 2002

Metabolic Reprogramming: A Cancer Hallmark Even Warburg Did Not Anticipate

Patrick S. Ward[1,2] and Craig B. Thompson[1,*]
[1]Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, NY 10065, USA
[2]Cell and Molecular Biology Graduate Group, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: craig@mskcc.org
DOI 10.1016/j.ccr.2012.02.014

Cancer metabolism has long been equated with aerobic glycolysis, seen by early biochemists as primitive and inefficient. Despite these early beliefs, the metabolic signatures of cancer cells are not passive responses to damaged mitochondria but result from oncogene-directed metabolic reprogramming required to support anabolic growth. Recent evidence suggests that metabolites themselves can be oncogenic by altering cell signaling and blocking cellular differentiation. No longer can cancer-associated alterations in metabolism be viewed as an indirect response to cell proliferation and survival signals. We contend that altered metabolism has attained the status of a core hallmark of cancer.

The propensity for proliferating cells to secrete a significant fraction of glucose carbon through fermentation was first elucidated in yeast. Otto Warburg extended these observations to mammalian cells, finding that proliferating ascites tumor cells converted the majority of their glucose carbon to lactate, even in oxygen-rich conditions. Warburg hypothesized that this altered metabolism was specific to cancer cells, and that it arose from mitochondrial defects that inhibited their ability to effectively oxidize glucose carbon to CO_2. An extension of this hypothesis was that dysfunctional mitochondria caused cancer (Koppenol et al., 2011). Warburg's seminal finding has been observed in a wide variety of cancers. These observations have been exploited clinically using ^{18}F-deoxyglucose positron emission tomography (FDG-PET). However, in contrast to Warburg's original hypothesis, damaged mitochondria are not at the root of the aerobic glycolysis exhibited by most tumor cells. Most tumor mitochondria are not defective in their ability to carry out oxidative phosphorylation. Instead, in proliferating cells, mitochondrial metabolism is reprogrammed to meet the challenges of macromolecular synthesis. This possibility was never considered by Warburg and his contemporaries.

Advances in cancer metabolism research over the last decade have enhanced our understanding of how aerobic glycolysis and other metabolic alterations observed in cancer cells support the anabolic requirements associated with cell growth and proliferation. It has become clear that anabolic metabolism is under complex regulatory control directed by growth-factor signal transduction in nontransformed cells. Yet despite these advances, the repeated refrain from traditional biochemists is that altered metabolism is merely an indirect phenomenon in cancer, a secondary effect that pales in importance to the activation of primary proliferation and survival signals (Hanahan and Weinberg, 2011). Most proto-oncogenes and tumor suppressor genes encode components of signal transduction pathways. Their roles in carcinogenesis have traditionally been attributed to their ability to regulate the cell cycle and sustain proliferative signaling while also helping cells evade growth suppression and/or cell death (Hanahan and Weinberg, 2011). But evidence for an alternative concept, that the primary functions of activated oncogenes and inactivated tumor suppressors are to reprogram cellular metabolism, has continued to build over the past several years. Evidence is also developing for the proposal that proto-oncogenes and tumor suppressors primarily evolved to regulate metabolism.

We begin this review by discussing how proliferative cell metabolism differs from quiescent cell metabolism on the basis of active metabolic reprogramming by proto-oncogenes and tumor suppressors. Much of this reprogramming depends on utilizing mitochondria as functional biosynthetic organelles. We then further develop the idea that altered metabolism is a primary feature selected for during tumorigenesis. Recent advances have demonstrated that altered metabolism in cancer extends beyond adaptations to meet the increased anabolic requirements of a growing and dividing cell. Changes in cancer cell metabolism can also influence cellular differentiation status, and in some cases these changes arise from oncogenic alterations in metabolic enzymes themselves.

Quiescent versus Proliferating Cells: Both Use Mitochondria, but to Different Ends

Most nonproliferating, differentiated cells depend on the efficiency of ATP production through oxidative phosphorylation to maintain their integrity. As a result, such cells metabolize glucose to pyruvate through glycolysis, and then completely oxidize most of this pyruvate to CO_2 through the tricarboxylic acid (TCA) cycle of the mitochondria, where oxygen is the final acceptor in an electron transport chain that generates an electrochemical gradient facilitating ATP production. The elucidation of the TCA cycle and how cells maximize ATP production to maintain themselves was one of the great discoveries of the last century.

In vivo, metazoan cells are surrounded by an abundance of nutrients. However, unlike prokaryotes or single-cell eukaryotes, animal cells are not cell autonomous for nutrient uptake. Instead, just to survive, metazoan cells compete for limiting levels of growth factors that direct nutrient uptake (Rathmell et al., 2000). To survive under such conditions, differentiated cells adopt a catabolic metabolism focused on maximizing the efficiency of ATP production from limited nutrients (Deberardinis

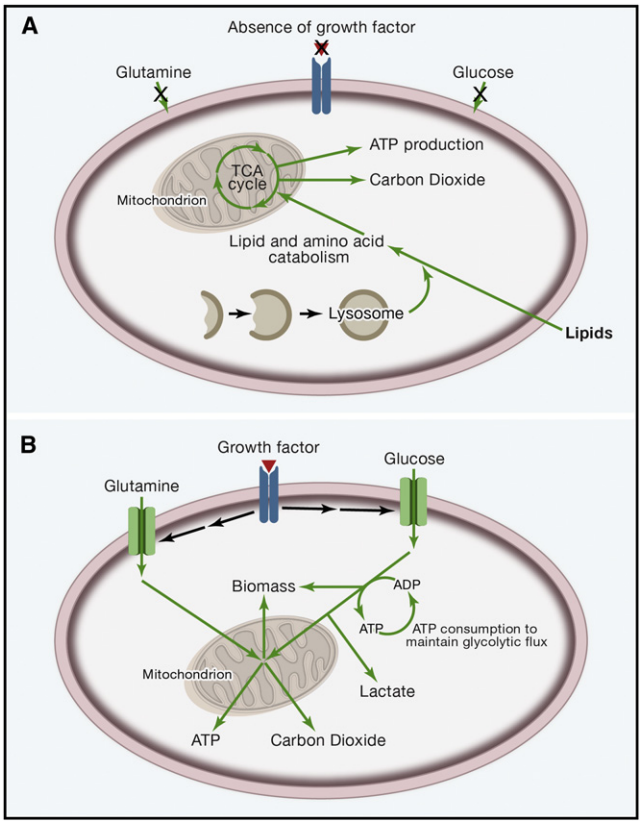

Figure 1. Metabolism in Quiescent versus Proliferating Cells: Both Use Mitochondria

(A) In the absence of instructional growth factor signaling, cells in multicellular organisms lack the ability to take up sufficient nutrients to maintain themselves. Neglected cells will undergo autophagy and catabolize amino acids and lipids through the TCA cycle, assuming sufficient oxygen is available. This oxidative metabolism maximizes ATP production.

(B) Cells that receive instructional growth factor signaling are directed to increase their uptake of nutrients, most notably glucose and glutamine. The increased nutrient uptake can then support the anabolic requirements of cell growth: mainly lipid, protein, and nucleotide synthesis (biomass). Excess carbon is secreted as lactate. Proliferating cells may also use strategies to decrease their ATP production while increasing their ATP consumption. These strategies maintain the ADP:ATP ratio necessary to sustain glycolytic flux. Green arrows represent metabolic pathways, while black arrows represent signaling.

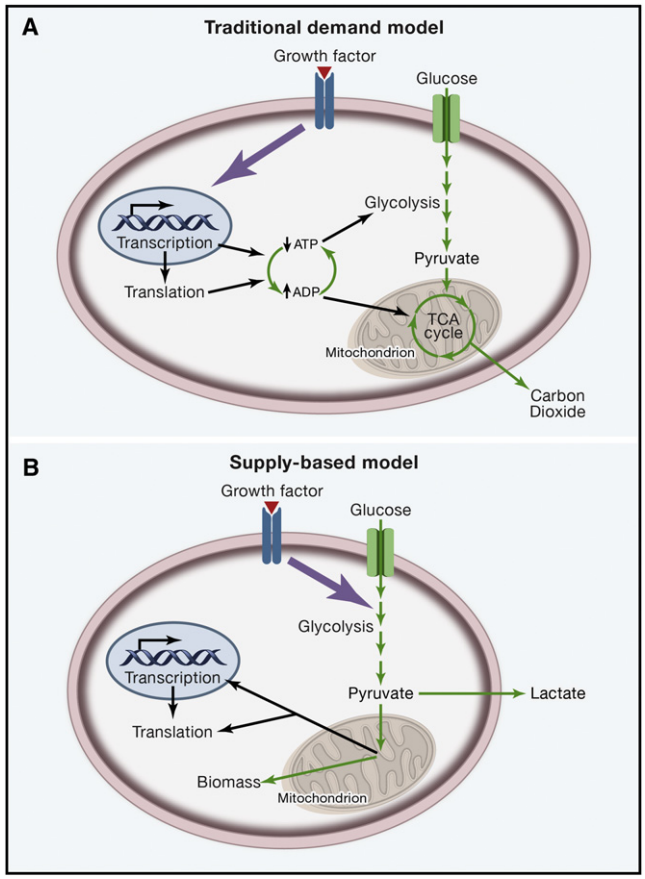

Figure 2. Metabolism Is a Direct, Not Indirect, Response to Growth Factor Signaling

(A) The traditional demand-based model of how metabolism is altered in proliferating cells. In response to growth factor signaling, increased transcription and translation consume free energy and decrease the ATP:ADP ratio. This leads to enhanced flux of glucose carbon through glycolysis and the TCA cycle for the purpose of producing more ATP.

(B) Supply-based model of how metabolism changes in proliferating cells. Growth factor signaling directly reprograms nutrient uptake and metabolism. Increased nutrient flux through glycolysis and the mitochondria in response to growth factor signaling is used for biomass production. Metabolism also impacts transcription and translation through mechanisms independent of ATP availability.

et al., 2006; Lum et al., 2005; Vander Heiden et al., 2009) (Figure 1A). In contrast, when growth factors are abundant, cells increase their nutrient uptake and adopt an anabolic metabolism (Bauer et al., 2004) (Figure 1B). As a consequence of intracellular abundance of nutrients, growth-factor-stimulated cells adapt to their largesse by initiating cell division in a manner analogous to that of single-cell eukaryotes exposed to nutrient-rich medium (Boer et al., 2010; Conlon and Raff, 2003; Fantes and Nurse, 1977). In cancer cells, the instructional signaling pathways downstream of growth factor receptors can be constitutively activated in the absence of extracellular growth factors.

Altered Metabolism Is a Direct Response to Growth-Factor Signaling

The traditional cancer model posits that the altered metabolism associated with cell proliferation occurs as a secondary response to cell cycle and proliferative signaling. In this model, the demand for free energy to sustain transcription and translation drives a decrease in the ATP:ADP ratio, leading to subsequent allosteric effects on rate-limiting metabolic enzymes (Figure 2A). While traditional allosteric regulation certainly occurs in proliferating cells, strong evidence now exists to support an alternative model. In this supply-based model, changes in metabolic fluxes occur in primary response to growth-factor signaling, independent of changes in ATP and other mechanisms worked out by early biochemists (Figure 2B). The reprogramming of cellular metabolism toward macromolecular synthesis is critical to supplying enough nucleotides, proteins, and lipids for a cell to double its total biomass and then divide to produce two daughter cells. In contrast to the catabolic metabolism of differentiated cells, this anabolic metabolism fundamental to cell growth and proliferation is not focused on maximizing ATP

yield. Rather than ATP, proliferating cells are in much greater need of reduced carbon and reduced nitrogen, as well as cytosolic NADPH for reductive biosynthetic reactions.

The recognition that proliferating cells do not maximize ATP production through mitochondrial oxidative phosphorylation has contributed to the continuing misconception that proliferating cells, particularly cancer cells, do not utilize mitochondria. In fact, most cancer cells and proliferating normal cells still derive a significant fraction of their ATP through oxidative phosphorylation. However, in proliferating cells, in contrast to quiescent cells, this oxidative phosphorylation-dependent production of ATP appears secondary to the use of mitochondrial enzymes in the synthesis of anabolic precursors.

PI3K/Akt/mTORC1 Activation: Driving Anabolic Metabolism and Tumorigenesis by Reprogramming Mitochondria

Activation of the PI3K/Akt pathway is perhaps the most common lesion in spontaneous human cancers. Activated PI3K/Akt leads to enhanced glucose uptake and glycolysis (Buzzai et al., 2005; Elstrom et al., 2004). Pivotal to this induction is increased glucose transporter expression on the cell surface, activation of hexokinase to capture glucose intracellularly through phosphorylation, and Akt-induced, phosphofructokinase-2-dependent allosteric activation of phosphofructokinase-1 to commit glucose to glycolytic metabolism (Deprez et al., 1997; Gottlob et al., 2001; Kohn et al., 1996; Rathmell et al., 2003). However, the PI3K/Akt pathway also promotes glucose carbon flux into biosynthetic pathways that rely upon functional mitochondrial metabolism (Figure 3). For example, fatty acid, cholesterol, and isoprenoid synthesis all require acetyl-CoA (Wakil et al., 1957). The pyruvate dehydrogenase (PDH) complex that converts glucose-derived pyruvate into acetyl-CoA is solely mitochondrial (Linn et al., 1969). Mitochondrial acetyl-CoA then cannot be directly exported to the cytoplasm but instead must first condense with oxaloacetate to form citrate through the activity of another exclusively mitochondrial enzyme, citrate synthase (Stern et al., 1952). Citrate can then be exported to the cytosol, where it can be converted back to acetyl-CoA by ATP-citrate lyase (ACL) (Srere, 1959). Akt facilitates this diversion of mitochondrial citrate from the TCA cycle to acetyl-CoA production by phosphorylating and activating ACL (Bauer et al., 2005; Berwick et al., 2002; Hatzivassiliou et al., 2005). RNAi knockdown or pharmacologic inhibition of ACL is particularly effective at decreasing the in vitro proliferation of cells with increased glucose uptake. ACL knockdown can also diminish Akt-driven tumorigenesis in vivo (Bauer et al., 2005; Hatzivassiliou et al., 2005). ACL's breakdown of citrate is also pivotal to preventing a cytosolic accumulation of citrate. Citrate is a major negative allosteric regulator of glycolysis (Stryer, 1995). Taken together, these findings demonstrate that the reprogramming of mitochondrial citrate metabolism is a central aspect of PI3K/Akt oncogenic activity.

Downstream of PI3K/Akt, the well-characterized cell growth regulator mTORC1 also has many effects intertwined with mitochondrial metabolism. mTORC1 is best known for enhancing protein synthesis. Several amino acid precursors are derived from the transamination of mitochondrial intermediates. Oxaloacetate can be transaminated to produce aspartate which can serve as a precursor for asparagine, and α-ketoglutarate can be transaminated to produce glutamate, which in turn can be converted to proline, arginine, and glutamine. Most cancers depend on these syntheses rather than exogenous supplies. This is consistent with how most tumors other than childhood leukemia are resistant to the effects of depleting the blood of asparagine through the intravenous use of L-asparaginase (Clarkson et al., 1970; Tallal et al., 1970). mTORC1 has also been shown to have direct effects on promoting mitochondrial biogenesis, in part via a transcriptional complex that promotes the function of PGC-1α (Bentzinger et al., 2008; Cunningham et al., 2007; Ramanathan and Schreiber, 2009; Schieke et al., 2006). Finally, a study isolating the cell-intrinsic consequences of mTORC1 activation demonstrated that SREBP-mediated de novo lipogenesis is a critical component of mTORC1-driven proliferation (Düvel et al., 2010). As discussed above, de novo lipogenesis in mammalian cells depends on mitochondrial citrate production.

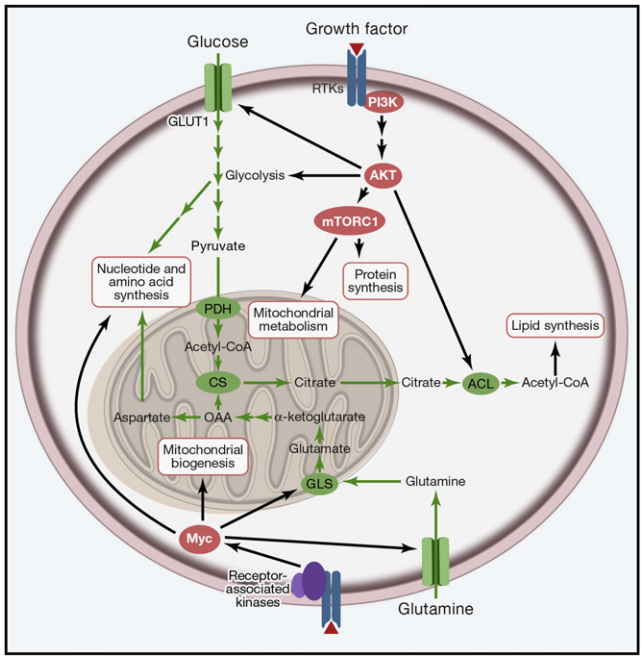

Figure 3. Alterations in Classic Oncogenes Directly Reprogram Cell Metabolism to Increase Nutrient Uptake and Biosynthesis

PI3K/Akt signaling downstream of receptor tyrosine kinase (RTK) activation increases glucose uptake through the transporter GLUT1, and increases flux through glycolysis. Branches of glycolytic metabolism contribute to nucleotide and amino acid synthesis. Akt also activates ATP-citrate lyase (ACL), promoting the conversion of mitochondria-derived citrate to acetyl-CoA for lipid synthesis. Mitochondrial citrate can be synthesized when glucose-derived acetyl-CoA, generated by pyruvate dehydrogenase (PDH), condenses with glutamine-derived oxaloacetate (OAA) via the activity of citrate synthase (CS). mTORC1 promotes protein synthesis and mitochondrial metabolism. Myc increases glutamine uptake and the conversion of glutamine into a mitochondrial carbon source by promoting the expression of the enzyme glutaminase (GLS). Myc also promotes mitochondrial biogenesis. In addition, Myc promotes nucleotide and amino acid synthesis, both through direct transcriptional regulation and through increasing the synthesis of mitochondrial metabolite precursors.

HIF-1-Mediated Inhibition of Carbon Flux into Mitochondria Can Be Antiproliferative

Notably, Düvel et al. (2010) found that the other major target of mTORC1 activation, hypoxia-inducible factor 1 (HIF-1), is not

critical for mTORC1-driven proliferation. This may seem surprising in light of HIF-1's often-cited ability to promote the enhanced glycolysis characteristic of cancer cells. However, HIF-1 activation has the additional effect of inhibiting mitochondrial metabolism of glucose carbon, in part by promoting the expression of pyruvate dehydrogenase kinase 1 (PDK1) to inhibit PDH activity (Kim et al., 2006; Papandreou et al., 2006). By diverting pyruvate into lactate, HIF-1 blocks glucose carbon incorporation into mitochondrial citrate which is critical for lipid synthesis (Lum et al., 2007). This block correlates with the antiproliferative effect of HIF-1 observed in hematopoietic and renal cells (Lum et al., 2007) and fits with recent genetic evidence of HIF-1 acting as a tumor suppressor in some cancers (Shen et al., 2011).

There are cancers that do exhibit decreased flux of glucose-derived pyruvate into the mitochondria relative to normal tissues. However, as will be discussed later in this review, these cancers still rely on mitochondrial metabolic flux. In place of oxidative metabolism of both glucose and glutamine, these cancers preferentially perform reductive and carboxylating biosynthetic reactions from glutamine carbon (Le et al., 2012; Metallo et al., 2012; Mullen et al., 2012; Wise et al., 2011).

Myc Activation Also Impacts Mitochondrial Metabolism

Like PI3K, Akt, and mTORC1, the Myc transcription factor has important metabolic roles beyond enhancing glycolysis. Myc promotes mitochondrial gene expression and mitochondrial biogenesis (Li et al., 2005). Oncogenic Myc has also been shown to promote the mitochondrial utilization of glutamine by enhancing the expression of glutaminase (GLS), which deamidates glutamine to glutamate. Cells expressing oncogenic Myc are glutamine-addicted and undergo apoptosis when glutamine is withdrawn from the culture medium. While the role of glutamine as a nitrogen donor is important for the proliferation of these cells, their viability depends on glutamine as a carbon source for mitochondrial metabolism (DeBerardinis et al., 2007; Fan et al., 2010; Gao et al., 2009; Wise et al., 2008; Yuneva et al., 2007). Recently, it was observed that the growth of tumor xenografts from Myc-expressing B cells can be impaired by pharmacological inhibition of GLS (Le et al., 2012). These data provide further evidence that reprogrammed glutamine metabolism is critical to the growth and survival of Myc-driven malignancies. Upstream of Myc, RhoGTPases have also been linked to the activation of GLS and glutamine dependence. Either siRNA knockdown or pharmacological inhibition of GLS can inhibit Rho-GTPase-induced transformation and proliferation (Wang et al., 2010).

Did Proto-Oncogenes and Tumor Suppressors Arise in Evolution as Components of Metabolic Regulation?

The weight of the evidence to date supports the concept that reprogramming of cellular metabolism is a primary and fundamental aspect of transformation resulting from mutations in proto-oncogenes and tumor suppressors. Proliferative metabolism is heavily dependent on the reprogramming of mitochondria to serve a synthetic rather than a degradative role. Metabolic changes associated with proliferating cells do not simply occur passively in response to damaged mitochondria or changes in ATP levels.

A related concept concerns the possibility that proto-oncogenes and tumor suppressors arose in evolution as components of metabolic regulation. Consistent with this hypothesis, activation of the tumor suppressor p53 has been shown to be critical for cell survival following glucose depletion (Jones et al., 2005). Subsequent reports have linked this metabolic stress response of p53 to increased fatty acid oxidation (Assaily et al., 2011; Zaugg et al., 2011). In tumors, the loss of p53 can enhance glycolysis and anabolic synthesis from glycolytic intermediates (Bensaad et al., 2006; Kondoh et al., 2005; Matoba et al., 2006). However, mitochondrial metabolism continues to be critical in cells with metabolic reprogramming arising from p53 loss. Treatment with the antidiabetic drug metformin, an inhibitor of complex 1 of the mitochondrial electron transport chain (El-Mir et al., 2000; Owen et al., 2000), is especially toxic to p53-deficient tumor cells (Buzzai et al., 2007).

Oncogenic mutations in proto-oncogenes can be selected for in tumor populations subjected to metabolic stress. Yun et al. (2009) showed that depriving colon carcinoma cells of glucose increased the rate at which activating mutations in Ras grew out. Surviving clones were better able to cope with limited glucose due to their upregulation of the transporter GLUT1. Some clones demonstrated *KRAS* mutations, and mutant KRAS was shown to upregulate GLUT1 expression and confer sensitivity to glycolytic inhibition. Importantly, increased glycolytic metabolism from activated Ras does not stem from defective mitochondrial pathways. Even for Ras-mediated tumorigenesis, the importance of intact mitochondrial oxidative metabolism has been confirmed in vivo (Guo et al., 2011; Weinberg et al., 2010).

Metabolic Enzymes Can Be Alternatively Spliced to Isoforms that Support Anabolic Growth

In addition to activating oncogenes like Ras, preferentially expressing specific isoforms of metabolic enzymes can provide cancer cells with a mechanism to select for metabolic alterations during tumorigenesis. For example, proliferating cells almost universally express the M2 isoform of pyruvate kinase M (PKM2). Pyruvate kinase is a glycolytic enzyme that converts phosphoenolpyruvate (PEP) to pyruvate, with concomitant generation of ATP. In contrast to the M1 isoform of pyruvate kinase (PKM1) that is the predominant isoform in most adult differentiated tissues, the PKM2 splice variant is the major isoform in embryonic tissues and in all cancer cells examined to date (Mazurek, 2011). Other significant genes for proliferative cell metabolism are also alternatively spliced. The phosphofructokinase/fructose-2,6-bisphosphatase B3 gene (*PFKFB3*) is highly expressed in human tumors and has six splice variants. Two splice variants predominate in high-grade astrocytoma and colon carcinoma and enhance glycolytic flux, while other splice variants are limited to low-grade tumors and normal tissues (Bando et al., 2005; Zscharnack et al., 2009). An alternatively spliced isoform of *GLS* may also be important for the mitochondrial glutamine metabolism of tumor cells (Cassago et al., 2012).

The most extensively characterized of these alternatively spliced metabolic enzymes remains pyruvate kinase. The preferential expression of PKM2 in proliferating cells suggested a protumorigenic role for this splice variant, and xenograft models

subsequently demonstrated that PKM2-expressing cells have a growth advantage in vivo compared with PKM1-expressing cells (Christofk et al., 2008a). However, in what might seem paradoxical for an isoform associated with proliferating and highly glycolytic cells, PKM2 has intrinsically lower enzymatic activity than PKM1. PKM2 is also uniquely sensitive to inhibition by tyrosine kinase signaling downstream of growth factor receptors, in contrast to PKM1 which is constitutively active (Christofk et al., 2008b; Hitosugi et al., 2009). Current evidence suggests that the decreased activity of PKM2 is selected to facilitate anabolic metabolism (Figure 4). With less rapid conversion of PEP to pyruvate, the accumulation of upstream glycolytic intermediates and subsequent shunting of these intermediates into anabolic pathways branching off glycolysis can result. These pathways include pyrimidine biosynthesis (Mazurek et al., 2001). They may also include the glycerol synthesis and serine/glycine synthesis pathways.

The Primary Role of PKM2 Expression in Proliferating Cells Is to Facilitate Anabolic Metabolism

Accompanying the resurgence of interest in the metabolic effects of PKM2 have been recent reports on "non-metabolic" functions of PKM2 (Luo et al., 2011; Yang et al., 2011). These studies are the latest variations on a theme from work spanning over a decade that have identified at least 11 proteins that bind to PKM2 (Garcia-Gonzalo et al., 2003; Le Mellay et al., 2002; Mazurek et al., 2001, 2007; Shimada et al., 2008; Siwko and Mochly-Rosen, 2007; Spoden et al., 2009; Williams et al., 1998; Wu et al., 2008; Zwerschke et al., 1999). Several of these are nuclear proteins, suggesting that PKM2 may, under certain conditions, translocate to the nucleus to fulfill nonmetabolic, putatively transcriptional functions (Hoshino et al., 2007; Ignacak and Stachurska, 2003; Lee et al., 2008; Spoden et al., 2008; Steták et al., 2007). However, other studies have added further strength to the concept that reduced pyruvate kinase enzymatic activity in the cytoplasm, leading to enhanced anabolic metabolism from glycolytic intermediates, is the primary role for PKM2 expression in proliferating cells. A recent study showed that in cancer cells, elevation of reactive oxygen species (ROS) can inactivate the active, tetrameric form of the cytosolic PKM2 enzyme (Anastasiou et al., 2011). Another report showed that PKM2 has a unique lysine residue which is acetylated in tumor cells, targeting it for degradation by chaperone-mediated autophagy (Lv et al., 2011). Both of these findings are consistent with PKM2 primarily acting as a glycolytic switch that can be rapidly inactivated in tumor cells by multiple mechanisms, all of which facilitate the shunting of glucose carbon/glycolytic intermediates into branching anabolic pathways.

A major paradox remaining with PKM2 is that cells expressing PKM2 produce more glucose-derived pyruvate than PKM1-expressing cells, despite having a form of the pyruvate kinase enzyme that is less active and more sensitive to inhibition. One way to get around the PKM2 bottleneck and maintain/enhance pyruvate production may be through a proposed alternative glycolytic pathway involving an enzymatic activity not yet purified that dephosphorylates PEP to pyruvate without the generation of ATP (Vander Heiden et al., 2010). Another answer to this paradox may emanate from the serine synthetic pathway. The decreased enzymatic activity of PKM2 can promote the accumulation of the 3-phosphoglycerate glycolytic intermediate that serves as the entry point for the serine synthetic pathway branch off glycolysis. The little-studied enzyme serine dehydratase can then directly convert serine to pyruvate. A third explanation may lie in the oscillatory activity of PKM2 from the inactive dimer to the active tetramer form. Regulatory inputs into PKM2 like tyrosine phosphorylation and ROS destabilize the tetrameric form of PKM2 (Anastasiou et al., 2011; Christofk et al., 2008b; Hitosugi et al., 2009), but other inputs present in glycolytic cancer cells like fructose-1,6-bisphosphate and serine can continually allosterically activate and/or promote reformation of the PKM2 tetramer (Ashizawa et al., 1991; Eigenbrodt et al., 1983). Thus, PKM2 may be continually switching from inactive to active forms in cells, resulting in an apparent upregulation of flux through anabolic glycolytic branching pathways while also maintaining reasonable net flux of glucose carbon through PEP to pyruvate. With such an oscillatory system, small changes in the levels of any of the above-mentioned PKM2 regulatory inputs can cause exquisite, rapid adjustments to glycolytic flux. This would be predicted to be advantageous for proliferating cells in the setting of variable extracellular nutrient availability. The capability for oscillatory regulation of PKM2 could also provide an explanation for why tumor cells do not select for altered glycolytic metabolism

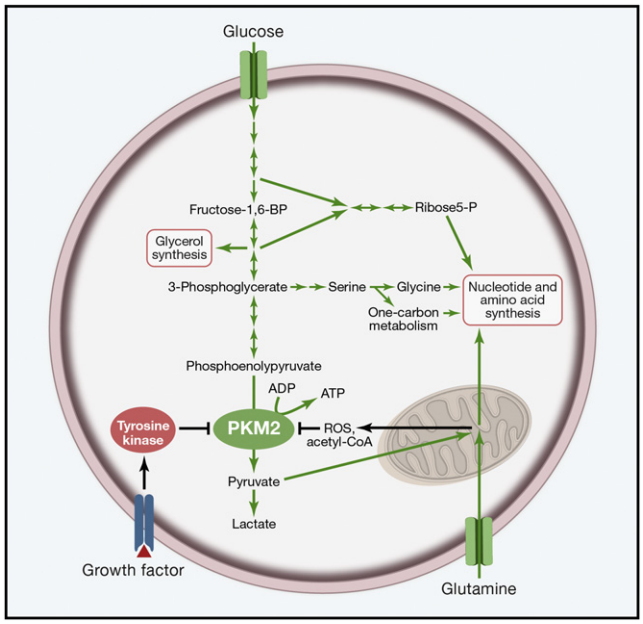

Figure 4. Pyruvate Kinase M2 Expression in Proliferating Cells Is Regulated by Signaling and Mitochondrial Metabolism to Facilitate Macromolecular Synthesis

Pyruvate kinase M2 (PKM2) is a less active isoform of the terminal glycolytic enzyme pyruvate kinase. It is also uniquely inhibited downstream of tyrosine kinase signaling. The decreased enzymatic activity of PKM2 in the cytoplasm promotes the accumulation of upstream glycolytic intermediates and their shunting into anabolic pathways. These pathways include the serine synthetic pathway that contributes to nucleotide and amino acid production. When mitochondrial metabolism is excessive, reactive oxygen species (ROS) from the mitochondria can feed back to inhibit PKM2 activity. Acetylation of PKM2, dependent on acetyl-CoA availability, may also promote PKM2 degradation and further contribute to increased flux through anabolic synthesis pathways branching off glycolysis.

upstream of PKM2 through deletions and/or loss-of-function mutations of other glycolytic enzymes.

Amplification of Metabolic Enzymes in Cancer

In addition to regulation by alternative splicing, the expression of metabolic enzymes may also be regulated by increases in gene copy number in cancer cells. The gene encoding hexokinase II, which catalyzes the first reaction of glycolysis converting glucose to glucose-6-phosphate, is amplified in hepatoma cells (Rempel et al., 1996), and fatty acid synthase has been reported to exhibit copy-number gain in prostate cancer (Shah et al., 2006). More recently, two independent groups have identified amplification of the gene encoding phosphoglycerate dehydrogenase in breast cancer and melanoma, resulting in increased flux through the serine/glycine synthesis pathway (Locasale et al., 2011; Possemato et al., 2011). As described above, the expression of PKM2 may provide an independent mechanism to enhance flux through the serine/glycine synthesis pathway. This enhanced flux may provide multiple metabolic advantages in addition to serine and glycine production. One proposed function is facilitating α-ketoglutarate production for mitochondrial metabolism (Possemato et al., 2011). Additional proposed functions include the generation of precursors for pyrimidine synthesis and the production of sarcosine, a glycine-derived metabolite linked to prostate cancer progression (Sreekumar et al., 2009; Zhang et al., 2012). Other important functions for flux through the serine synthesis pathway are likely.

Oncogenic Mutations in Metabolic Enzymes: The Discovery of IDH1 Mutations and the Oncometabolite 2-Hydroxyglutarate

While the preference for all tumor cells to express PKM2 versus PKM1 is striking and likely selected for to facilitate anabolic metabolism, PKM2 is present in most proliferating cells and is not specific to cancer (Mazurek, 2011). The strongest evidence to date that altered metabolism is selected for by cancer cells during tumorigenesis has come with the recent elucidation of somatic mutations in metabolic enzymes. Mutations in the cytosolic $NADP^+$-dependent isocitrate dehydrogenase 1 gene (*IDH1*) were first found to be recurrent in glioma and acute myeloid leukemia (AML) through whole-genome sequencing (Mardis et al., 2009; Parsons et al., 2008). The initially described mutations were remarkably selective for a specific arginine residue in the enzyme active site, R132. All mutations were missense, and all mutations were heterozygous with retention of the remaining wild-type *IDH1* allele. These characteristics suggested that the mutants acquired an altered function. Yet this contrasted with initial data demonstrating that the tumor-specific mutations in IDH1 and IDH2 resulted in loss of their normal enzymatic activity to interconvert isocitrate and α-ketoglutarate (Yan et al., 2009). The heterozygous nature of the mutations was then explained away with the report that IDH1 mutants can dominantly inhibit the wild-type IDH1 in cells (Zhao et al., 2009).

An alternative explanation came with the report that IDH1 mutations at R132 are not simply loss-of-function mutations for isocitrate and α-ketoglutarate interconversion, but also acquire a novel reductive activity to convert α-ketoglutarate to 2-hydroxyglutarate (2HG), a rare metabolite found only in trace amounts in mammalian cells under normal conditions (Dang et al., 2009). However, it still remained unclear whether 2HG was truly a pathogenic "oncometabolite" resulting from IDH1 mutation or was just the byproduct of a loss-of-function mutation. Whether 2HG production or the loss of IDH1 normal function played a more important role in tumorigenesis remained uncertain.

2HG Is a Biomarker and Common Feature of Both IDH1 and IDH2 Mutations

A potential answer to whether 2HG production was relevant to tumorigenesis arrived with the study of mutations in IDH2, the mitochondrial homolog of IDH1. Up to this point a small fraction of gliomas lacking IDH1 mutations were known to harbor mutations at IDH2 R172, the analogous residue to IDH1 R132 (Yan et al., 2009). However, given the rarity of these IDH2 mutations, they had not been characterized for 2HG production. The discovery of IDH2 R172 mutations in AML as well as glioma samples prompted the study of whether these mutations also conferred the reductive enzymatic activity to produce 2HG. Enzymatic assays and measurement of 2HG levels in primary AML samples confirmed that these IDH2 R172 mutations result in 2HG elevation (Gross et al., 2010; Ward et al., 2010).

It was then investigated whether the measurement of 2HG levels in primary tumor samples with unknown *IDH* mutation status could serve as a metabolite screening test for both cytosolic IDH1 and mitochondrial IDH2 mutations. AML samples with low to undetectable 2HG were subsequently sequenced and determined to be IDH1 and IDH2 wild-types, and several samples with elevated 2HG were found to have neomorphic mutations at either IDH1 R132 or IDH2 R172 (Gross et al., 2010). However, some 2HG-elevated AML samples lacked IDH1 R132 or IDH2 R172 mutations. When more comprehensive sequencing of *IDH1* and *IDH2* was performed, it was found that the common feature of this remaining subset of 2HG-elevated AMLs was another mutation in IDH2, occurring at R140 (Ward et al., 2010). This discovery provided additional evidence that 2HG production was the primary feature being selected for in tumors.

In addition to intensifying efforts to find the cellular targets of 2HG, the discovery of the 2HG-producing IDH1 and IDH2 mutations suggested that 2HG measurement might have clinical utility in diagnosis and disease monitoring. While much work is still needed in this area, serum 2HG levels have successfully correlated with IDH1 R132 mutations in AML, and recent data have suggested that ^{1}H magnetic resonance spectroscopy can be applied for 2HG detection in vivo for glioma (Andronesi et al., 2012; Choi et al., 2012; Gross et al., 2010; Pope et al., 2012). These methods may have advantages over relying on invasive-solid-tumor biopsies or isolating leukemic blast cells to obtain material for sequencing of *IDH1* and *IDH2*. Screening tumors and body fluids by 2HG status also has potentially increased applicability given the recent report that additional IDH mutations can produce 2HG (Ward et al., 2011). These additional alleles may account for the recently described subset of 2HG-elevated chondrosarcoma samples that lacked the most common *IDH1* or *IDH2* mutations but were not examined for other IDH alterations (Amary et al., 2011). Metabolite screening approaches can also distinguish neomorphic IDH mutations from SNPs and sequencing artifacts with no effect on IDH

enzyme activity, as well as from an apparently rare subset of loss-of-function, non-2HG-producing IDH mutations that may play a secondary tumorigenic role in altering cellular redox (Ward et al., 2011).

Metabolites as Oncogenes: Dysregulating Epigenetics and Cellular Differentiation

Consideration of the tumor subtypes where IDH mutations were most prevalent, the finding that IDH mutations occurred early in disease progression (Watanabe et al., 2009), and the lack of evidence that 2HG was acting as a mutagen (Mardis et al., 2009) led to investigation of whether 2HG accumulation might lead to impairment in cellular differentiation. Evidence in support of this hypothesis was first provided in 32D myeloid cells and in mouse primary bone marrow cells cultured ex vivo. In both cell types, the overexpression of IDH mutants blocked acquisition of mature myeloid markers while increasing the expression of stem-cell markers (Figueroa et al., 2010).

How could a small organic acid, 2HG, mediate such an effect? Numerous reports have now highlighted the ability of 2HG to inhibit several α-ketoglutarate-dependent dioxygenase enzymes (Figure 5). Contradictory claims exist regarding the ability of 2HG to inhibit the prolyl hydroxylase that targets HIF-1 for degradation (PHD2) to modulate HIF levels in cells. Several independent groups have failed to observe a direct link between 2HG and PHD2 inhibition. The weight of the evidence now suggests that regulation of HIF-1 stability through decreased PHD2 activity is not the primary effect of IDH mutations (Chowdhury et al., 2011; Dang et al., 2009; Jin et al., 2011; Mardis et al., 2009; Metellus et al., 2011; Williams et al., 2011). In contrast, multiple groups have found that IDH mutant expression and 2HG elevation can inhibit the TET family of enzymes that hydroxylate 5′-methylcytosine (Figueroa et al., 2010; Turcan et al., 2012; Xu et al., 2011). α-ketoglutarate-dependent TET activity produces 5′-hydroxymethylcytosine. This product can be an intermediate in either passive or active DNA demethylation through pathways that are still under active investigation. The biological relevance of TET inhibition by 2HG has strong genetic evidence: neomorphic mutations of *IDH1* or *IDH2* and *TET2* loss-of-function mutations were found to be mutually exclusive in a large AML cohort. Moreover, *TET2* mutant AML samples displayed an overlapping DNA hypermethylation signature with samples having *IDH1* or *IDH2* mutations, and shRNA knockdown of TET2 recapitulated the effect of IDH mutant overexpression on blocking hematopoietic cell differentiation (Figueroa et al., 2010).

The Oncometabolite 2HG Does More than Inhibit TET Activity and DNA Demethylation

Similar to hematologic malignancies, in gliomas and chondrosarcomas *IDH1* or *IDH2* mutations have been linked with altered DNA methylation profiles (Noushmehr et al., 2010; Pansuriya et al., 2011; Turcan et al., 2012). However, in gliomas and chondrosarcomas, there is no evidence of mutations in TET enzymes. This could potentially be due to the fact that redundant TET enzymes are expressed in glial and chondrosarcoma precursor cells, making it difficult to inactivate TET family function by mutation in such cells. Alternatively, other chromatin-modifying α-ketoglutarate-dependent dioxygenase enzymes may be inhibited by 2HG in these cells. Although one initial report implicated a broad array of enzymes that were affected by 2HG (Xu et al., 2011), a later report suggested greater specificity of 2HG for some specific Jumonji C domain histone demethylases (Chowdhury et al., 2011). Most recently, it has been reported that the mark most altered with stable, as opposed to transient, expression of IDH mutation is methylation at histone H3 lysine 9 (H3K9) (Lu et al., 2012). By studying the differentiation of 3T3-L1 fibroblasts into mature adipocytes, this study found that inhibition of H3K9 demethylation by 2HG, or siRNA knockdown of an H3K9 demethylase, was sufficient to block cell differentiation in the absence of changes in DNA methylation. This study also examined the effect of IDH mutation in immortalized astrocytes and found that a progressive accumulation of H3K9 methylation preceded the increase in DNA methylation. The

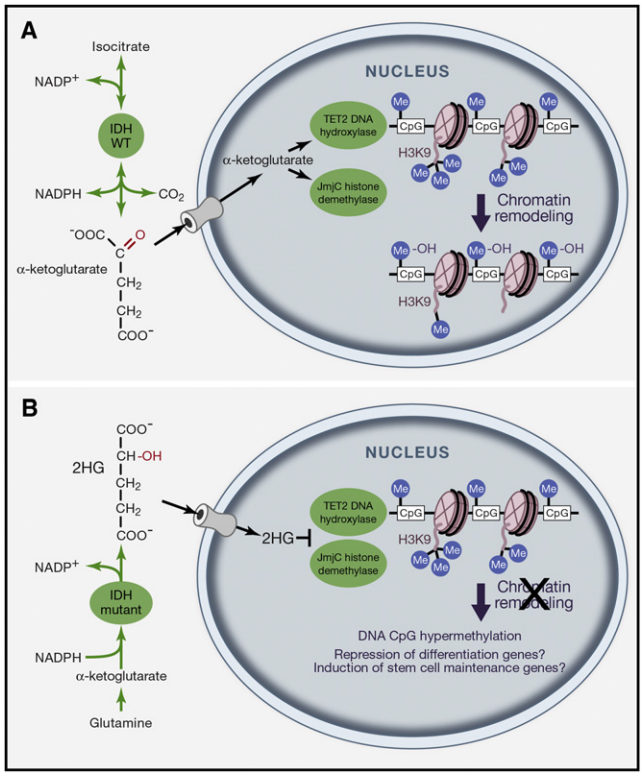

Figure 5. IDH1 and IDH2 Mutants Convert Glutamine Carbon to the Oncometabolite 2-Hydroxyglutarate to Dysregulate Epigenetics and Cell Differentiation

(A) α-ketoglutarate, produced in part by wild-type isocitrate dehydrogenase (IDH), can enter the nucleus and be used as a substrate for dioxygenase enzymes that modify epigenetic marks. These enzymes include the TET2 DNA hydroxylase enzyme, which converts 5-methylcytosine to 5-hydroxymethylcytosine, typically at CpG dinucleotides. 5-hydroxymethylcytosine may be an intermediate in either active or passive DNA demethylation. α-ketoglutarate is also a substrate for JmjC domain histone demethylase enzymes that demethylate lysine residues on histone tails.

(B) The common feature of cancer-associated mutations in cytosolic IDH1 and mitochondrial IDH2 is the acquisition of a neomorphic enzymatic activity. This activity converts glutamine-derived α-ketoglutarate to the oncometabolite 2HG. 2HG can competitively inhibit α-ketoglutarate-dependent enzymes like TET2 and the JmjC histone demethylases, thereby impairing normal epigenetic regulation. This results in altered histone methylation marks, in some cases DNA hypermethylation at CpG islands, and dysregulated cellular differentiation.

2HG-induced alterations in histone and DNA methylation are likely synergistic, and the precise relationship between these marks is the focus of continued investigation. For now, these findings provide further evidence that IDH mutation can impair differentiation in nontransformed cells from multiple cells of origin, and that this impairment is linked to 2HG-mediated epigenetic dysregulation.

Non-cell-autonomous effects of 2HG may also be important in some tumors. 2HG can inhibit the prolyl hydroxylase that regulates collagen synthesis (Xu et al., 2011). This inhibition could impact the tumor microenvironment and partly account for the diffuse nature of lower-grade gliomas. However, determination of whether this and other effects of 2HG are ultimately important for tumorigenesis will require careful future studies.

2HG Is Not the Only Oncometabolite

Will we find other novel oncometabolites like 2HG? We should consider basing the search for new oncometabolites on those metabolites already known to cause disease in pediatric inborn errors of metabolism (IEMs). 2HG exemplifies how advances in research on IEMs can inform research on cancer metabolism, and vice versa. Methods developed by those studying 2HG aciduria were used to demonstrate that *R*(−)-2HG (also known as D-2HG) is the exclusive 2HG stereoisomer produced by IDH1 and IDH2 mutants (Dang et al., 2009; Ward et al., 2010). Likewise, following the discovery of 2HG-producing *IDH2 R140* mutations in leukemia, researchers looked for and successfully found germline *IDH2 R140* mutations in D-2HG aciduria. *IDH2 R140* mutations now account for nearly half of all cases of this devastating disease (Kranendijk et al., 2010). While interest has surrounded 2HG due to its apparent novelty as a metabolite not found in normal nondiseased cells, there are situations where 2HG appears in the absence of metabolic enzyme mutations. For example, in human cells proliferating in hypoxia, α-ketoglutarate can accumulate and be metabolized through an enhanced reductive activity of wild-type IDH2 in the mitochondria, leading to 2HG accumulation in the absence of IDH mutation (Wise et al., 2011). The ability of 2HG to alter epigenetics may reflect its evolutionary ancient status as a signal for elevated glutamine/glutamate metabolism and/or oxygen deficiency.

With this broadened view of what constitutes an oncometabolite, one could argue that the discoveries of two other oncometabolites, succinate and fumarate, preceded that of 2HG. Loss-of-function mutations in the TCA cycle enzymes succinate dehydrogenase (SDH) and fumarate hydratase (FH) have been known for several years to occur in pheochromocytoma, paraganglioma, leiomyoma, and renal carcinoma. It was initially hypothesized that these mutations contribute to cancer through mitochondrial damage producing elevated ROS (Eng et al., 2003). However, potential tumorigenic effects were soon linked to the elevated levels of succinate and fumarate arising from loss of SDH function and FH function, respectively. Succinate was initially found to impair PHD2, the α-ketoglutarate-dependent enzyme regulating HIF stability, through product inhibition (Selak et al., 2005). Subsequent work confirmed that fumarate could inhibit PHD2 (Isaacs et al., 2005) and that succinate could also inhibit the related enzyme PHD3 (Lee et al., 2005). These observations linked the elevated HIF levels observed in SDH- and FH-deficient tumors to the activity of the succinate and fumarate metabolites. Recent work has suggested that fumarate may have other important roles that predominate in FH deficiency. For example, fumarate can modify cysteine residues to inhibit a negative regulator of the Nrf2 transcription factor. This posttranslational modification leads to the upregulation of antioxidant response genes (Adam et al., 2011; Ooi et al., 2011).

There are still many unanswered questions regarding the biology of SDH- and FH-deficient tumors. In light of the emerging epigenetic effects of 2HG, it is intriguing that succinate has been shown to alter histone demethylase activity in yeast (Smith et al., 2007). Perhaps elevated succinate and fumarate resulting from SDH and FH mutations can promote tumorigenesis in part through epigenetic modulation.

Textbook Biochemical Pathways Often Do Not Apply to Proliferating Cells

What has received little appreciation since the work of Krebs and his colleagues is that for many proliferating cells, the major problem is not how to maximize ATP yield, but rather how to maximize the flux of carbon into macromolecular synthetic pathways. In fact, it was first demonstrated in 1973 that glycolysis in proliferating cells is limited by the rate of ATP consumption and not ATP production, as glycolytic enzymes can be inhibited when ATP levels are high (Scholnick et al., 1973). Recent work has revisited how proliferating cells maintain glycolytic flux by either minimizing ATP production or enhancing ATP consumption. The proposed alternative glycolytic pathway to get around the PKM2 bottleneck may be one way to accomplish this (Vander Heiden et al., 2010). In this alternative pathway, the high-energy phosphate of PEP is transferred not to ADP but instead to a histidine residue on an upstream glycolytic enzyme. This alternative transfer therefore decouples the PEP→pyruvate conversion from ATP production. Another study found that cells with activated PI3K/Akt upregulate the activity of ENTPD5, an endoplasmic reticulum enzyme involved in glycosylation reactions and linked to ATP hydrolysis (Fang et al., 2010).

One pathway that supports cell proliferation and is not found in most textbooks is the ability of TCA-cycle enzymes to facilitate reductive carboxylation rather than oxidative metabolism. While this may seem heretical, IDH-dependent reductive carboxylation of α-ketoglutarate to isocitrate was described in early metabolic literature (Ochoa, 1948) and has been investigated since then as a way to produce citrate and fatty acids from α-ketoglutarate derived from glutamine (Holleran et al., 1995; Ward et al., 2010; Yoo et al., 2008). Recently, three independent reports implicated IDH-dependent reductive carboxylation as playing a particularly important role in proliferating cells that exhibit decreased flux of glucose-derived pyruvate into the mitochondria. This occurs in hypoxic cells or in cells harboring defects in mitochondrial oxidative phosphorylation (Metallo et al., 2012; Mullen et al., 2012; Wise et al., 2011). There are conflicting conclusions over whether the reductive flux of glutamine-derived carbon is primarily dependent upon cytosolic IDH1 or mitochondrial IDH2. These findings could all be correct if a mitochondrial-cytosolic NADPH shuttle exists using these enzymes (Figure 6). Although NADPH mitochondrial-cytosolic shuttles have not been previously described, it would allow high-energy electrons from NADPH in one compartment to be donated to α-ketoglutarate through reductive carboxylation and then transported to the other compartment

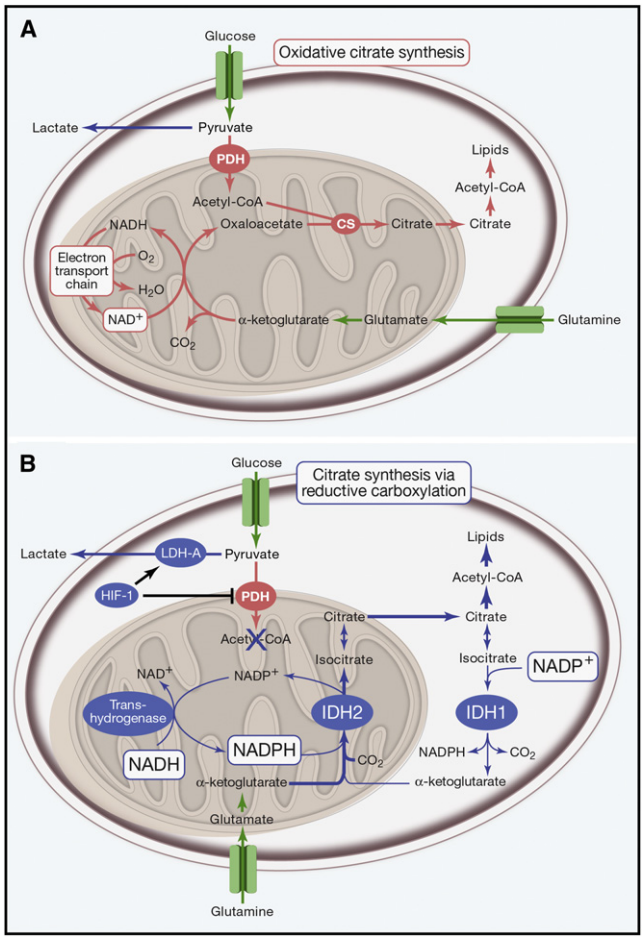

Figure 6. Hypoxia and HIF-1 Activation Promote an Alternative Pathway for Citrate Synthesis through Reductive Metabolism of Glutamine

(A) In proliferating cells under normoxic conditions, citrate is synthesized from both glucose and glutamine. Glucose carbon provides acetyl-CoA through the activity of PDH. Glutamine carbon provides oxaloacetate through oxidative mitochondrial metabolism dependent on NAD^+. Glucose-derived acetyl-CoA and glutamine-derived oxaloacetate condense to form citrate via the activity of citrate synthase (CS). Citrate can be exported to the cytosol for lipid synthesis. (B) In cells proliferating in hypoxia and/or with HIF-1 activation, glucose is diverted away from mitochondrial acetyl-CoA and citrate production. Citrate can be maintained through an alternative pathway of reductive carboxylation, which we propose to rely on reverse flux of glutamine-derived α-ketoglutarate through IDH2. This reverse flux in the mitochondria would promote electron export from the mitochondria when the activity of the electron transport chain is inhibited because of the lack of oxygen as an electron acceptor. Mitochondrial reverse flux can be accomplished by NADH conversion to NADPH by mitochondrial transhydrogenase and the resulting NADPH use in α-ketoglutarate carboxylation. When citrate/isocitrate is exported to the cytosol, some may be metabolized in the oxidative direction by IDH1 and contribute to a shuttle that produces cytosolic NADPH.

in the form of isocitrate/citrate for oxidation and regeneration of α-ketoglutarate and NADPH. Further work is needed to test this concept, but knowledge regarding cellular redox suggests the reductive flux is likely to occur in the mitochondria. Mitochondria have a high NADH:NAD^+ ratio, particularly in hypoxia (Chance and Thorell, 1959; Frezza et al., 2011), and NADH can be converted to NADPH within the mitochondria via a transhydrogenase that is absent from the cytosol (Rydström, 2006).

Unlike ATP, cytosolic NADPH might be limiting for cell proliferation. It is critical for providing reducing equivalents for fatty acid and cholesterol biosynthesis, as well as for modulating oxidative stress. Historically, the oxidative pentose phosphate pathway, branching off glycolysis at glucose-6-phosphate dehydrogenase (G6PD), has been considered the major NADPH-producing pathway. However, we offer that the repeated human experiment should be considered: millions of men are affected by the X-linked disorder of G6PD deficiency, yet cohort studies have not detected a decrease in cancer incidence in G6PD-deficient men (Cocco et al., 1998; Ferraris et al., 1988), and smaller case control studies have also failed to demonstrate a relationship (Forteleoni et al., 1988; Pisano et al., 1991). These studies focused on men with the Mediterranean variant of *G6PD*, an allele demonstrating severe enzyme deficiency with less than 10% of normal activity. The relationship between other disease-associated G6PD variants and cancer has not been rigorously tested. Notably, Ferraris et al. also examined females with mosaicism for the Mediterranean variant of *G6PD* (due to random X inactivation) who developed clonal hematological disorders. Neoplastic clones did not demonstrate preferential expression of the wild-type G6PD allele. Collectively, these data raise the possibility that significant flux through G6PD to generate NADPH is not necessarily critical for cell proliferation or tumorigenesis. While this hypothesis deserves further study, it would account for the observed propensity of tumor cells to synthesize pentose phosphates through a G6PD-independent pathway (Boros et al., 2000; Zhao et al., 2010). It would also fit with the importance of glucose carbon being metabolized through other branches of glycolytic metabolism, including serine synthesis (as discussed above) and the hexosamine pathway (Wellen et al., 2010). However, exactly which alternative NADPH-generating pathways are most important remain unclear. Further work in this area would benefit from improved methods for measuring NADPH.

Challenges Ahead for Studying the Metabolic Hallmarks of Cancer

Despite rapid technological advances in studying cell metabolism, we remain unable to reliably distinguish cytosolic metabolites from those in the mitochondria and other compartments. Current fractionation methods often lead to metabolite leakage. Even within one subcellular compartment, there may be distinct pools of metabolites resulting from channeling between metabolic enzymes. A related challenge lies in the quantitative measurement of metabolic flux, i.e., measuring the movement of carbon, nitrogen, and other atoms through metabolic pathways rather than simply measuring the steady-state levels of individual metabolites. While critical fluxes have been quantified in cultured cancer cells, and methods for these analyses continue to improve (DeBerardinis et al., 2007; Mancuso et al., 2004; Yuan et al., 2008), many obstacles remain, such as cellular compartmentalization and the reliance of most cell culture on complex, incompletely defined media.

Non-cell-autonomous effects of tumor metabolism represent another emerging challenge. For example, lactate produced by tumor cells can acidify the surrounding microenvironment and potentially promote tumor invasion, and some tumors may exhibit a symbiosis between lactate-producing cells and

lactate-consuming cells (Sonveaux et al., 2008). In addition, fatty acid exchange between omental adipocytes and ovarian carcinoma cells has been documented (Nieman et al., 2011). These areas appear ripe for further study.

Over the past decade, the study of metabolism has returned to its rightful place at the forefront of cancer research. Although Warburg was wrong about mitochondria, he was prescient in his focus on metabolism. Data now support the concepts that altered metabolism results from active reprogramming by altered oncogenes and tumor suppressors, and that metabolic adaptations can be clonally selected during tumorigenesis. Altered metabolism should now be considered a core hallmark of cancer. There is much work to be done.

ACKNOWLEDGMENTS

We thank members of the Thompson Laboratory, particularly Chao Lu and Lydia Finley, and Anthony Mancuso for helpful discussions. The Thompson Laboratory is supported in part by grants from the NCI and NIH. P.S.W. is also supported in part by the University of Pennsylvania Medical Scientist Training Program. C.B.T. has multiple patent applications relevant to the issues discussed in this review. These patent applications are assigned to the University of Pennsylvania. C.B.T. is a co-founder of Agios Pharmaceuticals and a member of the board of directors of Merck.

REFERENCES

Adam, J., Hatipoglu, E., O'Flaherty, L., Ternette, N., Sahgal, N., Lockstone, H., Baban, D., Nye, E., Stamp, G.W., Wolhuter, K., et al. (2011). Cancer Cell *20*, 524–537.

Amary, M.F., Damato, S., Halai, D., Eskandarpour, M., Berisha, F., Bonar, F., McCarthy, S., Fantin, V.R., Straley, K.S., Lobo, S., et al. (2011). Nat. Genet. *43*, 1262–1265.

Anastasiou, D., Poulogiannis, G., Asara, J.M., Boxer, M.B., Jiang, J.K., Shen, M., Bellinger, G., Sasaki, A.T., Locasale, J.W., Auld, D.S., et al. (2011). Science *334*, 1278–1283.

Andronesi, O.C., Kim, G.S., Gerstner, E., Batchelor, T., Tzika, A.A., Fantin, V.R., Vander Heiden, M.G., and Sorensen, A.G. (2012). Sci. Transl. Med. *4*, 116ra4.

Ashizawa, K., Willingham, M.C., Liang, C.M., and Cheng, S.Y. (1991). J. Biol. Chem. *266*, 16842–16846.

Assaily, W., Rubinger, D.A., Wheaton, K., Lin, Y., Ma, W., Xuan, W., Brown-Endres, L., Tsuchihara, K., Mak, T.W., and Benchimol, S. (2011). Mol. Cell *44*, 491–501.

Bando, H., Atsumi, T., Nishio, T., Niwa, H., Mishima, S., Shimizu, C., Yoshioka, N., Bucala, R., and Koike, T. (2005). Clin. Cancer Res. *11*, 5784–5792.

Bauer, D.E., Harris, M.H., Plas, D.R., Lum, J.J., Hammerman, P.S., Rathmell, J.C., Riley, J.L., and Thompson, C.B. (2004). FASEB J. *18*, 1303–1305.

Bauer, D.E., Hatzivassiliou, G., Zhao, F., Andreadis, C., and Thompson, C.B. (2005). Oncogene *24*, 6314–6322.

Bensaad, K., Tsuruta, A., Selak, M.A., Vidal, M.N., Nakano, K., Bartrons, R., Gottlieb, E., and Vousden, K.H. (2006). Cell *126*, 107–120.

Bentzinger, C.F., Romanino, K., Cloëtta, D., Lin, S., Mascarenhas, J.B., Oliveri, F., Xia, J., Casanova, E., Costa, C.F., Brink, M., et al. (2008). Cell Metab. *8*, 411–424.

Berwick, D.C., Hers, I., Heesom, K.J., Moule, S.K., and Tavare, J.M. (2002). J. Biol. Chem. *277*, 33895–33900.

Boer, V.M., Crutchfield, C.A., Bradley, P.H., Botstein, D., and Rabinowitz, J.D. (2010). Mol. Biol. Cell *21*, 198–211.

Boros, L.G., Torday, J.S., Lim, S., Bassilian, S., Cascante, M., and Lee, W.N. (2000). Cancer Res. *60*, 1183–1185.

Buzzai, M., Bauer, D.E., Jones, R.G., Deberardinis, R.J., Hatzivassiliou, G., Elstrom, R.L., and Thompson, C.B. (2005). Oncogene *24*, 4165–4173.

Buzzai, M., Jones, R.G., Amaravadi, R.K., Lum, J.J., DeBerardinis, R.J., Zhao, F., Viollet, B., and Thompson, C.B. (2007). Cancer Res. *67*, 6745–6752.

Cassago, A., Ferreira, A.P., Ferreira, I.M., Fornezari, C., Gomes, E.R., Greene, K.S., Pereira, H.M., Garratt, R.C., Dias, S.M., and Ambrosio, A.L. (2012). Proc. Natl. Acad. Sci. USA *109*, 1092–1097.

Chance, B., and Thorell, B. (1959). J. Biol. Chem. *234*, 3044–3050.

Choi, C., Ganji, S.K., Deberardinis, R.J., Hatanpaa, K.J., Rakheja, D., Kovacs, Z., Yang, X.L., Mashimo, T., Raisanen, J.M., Marin-Valencia, I., et al. (2012). Nat Med. Published online January 26, 2012. 10.1038/nm.2682.

Chowdhury, R., Yeoh, K.K., Tian, Y.M., Hillringhaus, L., Bagg, E.A., Rose, N.R., Leung, I.K., Li, X.S., Woon, E.C., Yang, M., et al. (2011). EMBO Rep. *12*, 463–469.

Christofk, H.R., Vander Heiden, M.G., Harris, M.H., Ramanathan, A., Gerszten, R.E., Wei, R., Fleming, M.D., Schreiber, S.L., and Cantley, L.C. (2008a). Nature *452*, 230–233.

Christofk, H.R., Vander Heiden, M.G., Wu, N., Asara, J.M., and Cantley, L.C. (2008b). Nature *452*, 181–186.

Clarkson, B., Krakoff, I., Burchenal, J., Karnofsky, D., Golbey, R., Dowling, M., Oettgen, H., and Lipton, A. (1970). Cancer *25*, 279–305.

Cocco, P., Todde, P., Fornera, S., Manca, M.B., Manca, P., and Sias, A.R. (1998). Blood *91*, 706–709.

Conlon, I., and Raff, M. (2003). J. Biol. *2*, 7.

Cunningham, J.T., Rodgers, J.T., Arlow, D.H., Vazquez, F., Mootha, V.K., and Puigserver, P. (2007). Nature *450*, 736–740.

Dang, L., White, D.W., Gross, S., Bennett, B.D., Bittinger, M.A., Driggers, E.M., Fantin, V.R., Jang, H.G., Jin, S., Keenan, M.C., et al. (2009). Nature *462*, 739–744.

Deberardinis, R.J., Lum, J.J., and Thompson, C.B. (2006). J. Biol. Chem. *281*, 37372–37380.

DeBerardinis, R.J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S., and Thompson, C.B. (2007). Proc. Natl. Acad. Sci. USA *104*, 19345–19350.

Deprez, J., Vertommen, D., Alessi, D.R., Hue, L., and Rider, M.H. (1997). J. Biol. Chem. *272*, 17269–17275.

Düvel, K., Yecies, J.L., Menon, S., Raman, P., Lipovsky, A.I., Souza, A.L., Triantafellow, E., Ma, Q., Gorski, R., Cleaver, S., et al. (2010). Mol. Cell *39*, 171–183.

Eigenbrodt, E., Leib, S., Krämer, W., Friis, R.R., and Schoner, W. (1983). Biomed. Biochim. Acta *42*, S278–S282.

El-Mir, M.Y., Nogueira, V., Fontaine, E., Avéret, N., Rigoulet, M., and Leverve, X. (2000). J. Biol. Chem. *275*, 223–228.

Elstrom, R.L., Bauer, D.E., Buzzai, M., Karnauskas, R., Harris, M.H., Plas, D.R., Zhuang, H., Cinalli, R.M., Alavi, A., Rudin, C.M., and Thompson, C.B. (2004). Cancer Res. *64*, 3892–3899.

Eng, C., Kiuru, M., Fernandez, M.J., and Aaltonen, L.A. (2003). Nat. Rev. Cancer *3*, 193–202.

Fan, Y., Dickman, K.G., and Zong, W.X. (2010). J. Biol. Chem. *285*, 7324–7333.

Fang, M., Shen, Z., Huang, S., Zhao, L., Chen, S., Mak, T.W., and Wang, X. (2010). Cell *143*, 711–724.

Fantes, P., and Nurse, P. (1977). Exp. Cell Res. *107*, 377–386.

Ferraris, A.M., Broccia, G., Meloni, T., Forteleoni, G., and Gaetani, G.F. (1988). Am. J. Hum. Genet. *42*, 516–520.

Figueroa, M.E., Abdel-Wahab, O., Lu, C., Ward, P.S., Patel, J., Shih, A., Li, Y., Bhagwat, N., Vasanthakumar, A., Fernandez, H.F., et al. (2010). Cancer Cell *18*, 553–567.

Forteleoni, G., Argiolas, L., Farris, A., Ferraris, A.M., Gaetani, G.F., and Meloni, T. (1988). Tumori *74*, 665–667.

Frezza, C., Zheng, L., Tennant, D.A., Papkovsky, D.B., Hedley, B.A., Kalna, G., Watson, D.G., and Gottlieb, E. (2011). PLoS ONE *6*, e24411.

Gao, P., Tchernyshyov, I., Chang, T.C., Lee, Y.S., Kita, K., Ochi, T., Zeller, K.I., De Marzo, A.M., Van Eyk, J.E., Mendell, J.T., and Dang, C.V. (2009). Nature *458*, 762–765.

Garcia-Gonzalo, F.R., Cruz, C., Muñoz, P., Mazurek, S., Eigenbrodt, E., Ventura, F., Bartrons, R., and Rosa, J.L. (2003). FEBS Lett. *539*, 78–84.

Gottlob, K., Majewski, N., Kennedy, S., Kandel, E., Robey, R.B., and Hay, N. (2001). Genes Dev. *15*, 1406–1418.

Gross, S., Cairns, R.A., Minden, M.D., Driggers, E.M., Bittinger, M.A., Jang, H.G., Sasaki, M., Jin, S., Schenkein, D.P., Su, S.M., et al. (2010). J. Exp. Med. *207*, 339–344.

Guo, J.Y., Chen, H.Y., Mathew, R., Fan, J., Strohecker, A.M., Karsli-Uzunbas, G., Kamphorst, J.J., Chen, G., Lemons, J.M., Karantza, V., et al. (2011). Genes Dev. *25*, 460–470.

Hanahan, D., and Weinberg, R.A. (2011). Cell *144*, 646–674.

Hatzivassiliou, G., Zhao, F., Bauer, D.E., Andreadis, C., Shaw, A.N., Dhanak, D., Hingorani, S.R., Tuveson, D.A., and Thompson, C.B. (2005). Cancer Cell *8*, 311–321.

Hitosugi, T., Kang, S., Vander Heiden, M.G., Chung, T.W., Elf, S., Lythgoe, K., Dong, S., Lonial, S., Wang, X., Chen, G.Z., et al. (2009). Sci. Signal. *2*, ra73.

Holleran, A.L., Briscoe, D.A., Fiskum, G., and Kelleher, J.K. (1995). Mol. Cell. Biochem. *152*, 95–101.

Hoshino, A., Hirst, J.A., and Fujii, H. (2007). J. Biol. Chem. *282*, 17706–17711.

Ignacak, J., and Stachurska, M.B. (2003). Comp. Biochem. Physiol. B Biochem. Mol. Biol. *134*, 425–433.

Isaacs, J.S., Jung, Y.J., Mole, D.R., Lee, S., Torres-Cabala, C., Chung, Y.L., Merino, M., Trepel, J., Zbar, B., Toro, J., et al. (2005). Cancer Cell *8*, 143–153.

Jin, G., Reitman, Z.J., Spasojevic, I., Batinic-Haberle, I., Yang, J., Schmidt-Kittler, O., Bigner, D.D., and Yan, H. (2011). PLoS ONE *6*, e16812.

Jones, R.G., Plas, D.R., Kubek, S., Buzzai, M., Mu, J., Xu, Y., Birnbaum, M.J., and Thompson, C.B. (2005). Mol. Cell *18*, 283–293.

Kim, J.W., Tchernyshyov, I., Semenza, G.L., and Dang, C.V. (2006). Cell Metab. *3*, 177–185.

Kohn, A.D., Summers, S.A., Birnbaum, M.J., and Roth, R.A. (1996). J. Biol. Chem. *271*, 31372–31378.

Kondoh, H., Lleonart, M.E., Gil, J., Wang, J., Degan, P., Peters, G., Martinez, D., Carnero, A., and Beach, D. (2005). Cancer Res. *65*, 177–185.

Koppenol, W.H., Bounds, P.L., and Dang, C.V. (2011). Nat. Rev. Cancer *11*, 325–337.

Kranendijk, M., Struys, E.A., van Schaftingen, E., Gibson, K.M., Kanhai, W.A., van der Knaap, M.S., Amiel, J., Buist, N.R., Das, A.M., de Klerk, J.B., et al. (2010). Science *330*, 336.

Le, A., Lane, A.N., Hamaker, M., Bose, S., Gouw, A., Barbi, J., Tsukamoto, T., Rojas, C.J., Slusher, B.S., Zhang, H., et al. (2012). Cell Metab. *15*, 110–121.

Le Mellay, V., Houben, R., Troppmair, J., Hagemann, C., Mazurek, S., Frey, U., Beigel, J., Weber, C., Benz, R., Eigenbrodt, E., and Rapp, U.R. (2002). Adv. Enzyme Regul. *42*, 317–332.

Lee, J., Kim, H.K., Han, Y.M., and Kim, J. (2008). Int. J. Biochem. Cell Biol. *40*, 1043–1054.

Lee, S., Nakamura, E., Yang, H., Wei, W., Linggi, M.S., Sajan, M.P., Farese, R.V., Freeman, R.S., Carter, B.D., Kaelin, W.G., Jr., and Schlisio, S. (2005). Cancer Cell *8*, 155–167.

Li, F., Wang, Y., Zeller, K.I., Potter, J.J., Wonsey, D.R., O'Donnell, K.A., Kim, J.W., Yustein, J.T., Lee, L.A., and Dang, C.V. (2005). Mol. Cell. Biol. *25*, 6225–6234.

Linn, T.C., Pettit, F.H., and Reed, L.J. (1969). Proc. Natl. Acad. Sci. USA *62*, 234–241.

Locasale, J.W., Grassian, A.R., Melman, T., Lyssiotis, C.A., Mattaini, K.R., Bass, A.J., Heffron, G., Metallo, C.M., Muranen, T., Sharfi, H., et al. (2011). Nat. Genet. *43*, 869–874.

Lu, C., Ward, P.S., Kapoor, G.S., Rohle, D., Turcan, S., Abdel-Wahab, O., Edwards, C., Khanin, R., Figueroa, M.E., Melnick, A., et al. (2012). Nature. Published online February 15, 2012. 10.1038/nature10860.

Lum, J.J., Bauer, D.E., Kong, M., Harris, M.H., Li, C., Lindsten, T., and Thompson, C.B. (2005). Cell *120*, 237–248.

Lum, J.J., Bui, T., Gruber, M., Gordan, J.D., DeBerardinis, R.J., Covello, K.L., Simon, M.C., and Thompson, C.B. (2007). Genes Dev. *21*, 1037–1049.

Luo, W., Hu, H., Chang, R., Zhong, J., Knabel, M., O'Meally, R., Cole, R.N., Pandey, A., and Semenza, G.L. (2011). Cell *145*, 732–744.

Lv, L., Li, D., Zhao, D., Lin, R., Chu, Y., Zhang, H., Zha, Z., Liu, Y., Li, Z., Xu, Y., et al. (2011). Mol. Cell *42*, 719–730.

Mancuso, A., Beardsley, N.J., Wehrli, S., Pickup, S., Matschinsky, F.M., and Glickson, J.D. (2004). Biotechnol. Bioeng. *87*, 835–848.

Mardis, E.R., Ding, L., Dooling, D.J., Larson, D.E., McLellan, M.D., Chen, K., Koboldt, D.C., Fulton, R.S., Delehaunty, K.D., McGrath, S.D., et al. (2009). N. Engl. J. Med. *361*, 1058–1066.

Matoba, S., Kang, J.G., Patino, W.D., Wragg, A., Boehm, M., Gavrilova, O., Hurley, P.J., Bunz, F., and Hwang, P.M. (2006). Science *312*, 1650–1653.

Mazurek, S. (2011). Int. J. Biochem. Cell Biol. *43*, 969–980.

Mazurek, S., Zwerschke, W., Jansen-Dürr, P., and Eigenbrodt, E. (2001). Biochem. J. *356*, 247–256.

Mazurek, S., Drexler, H.C., Troppmair, J., Eigenbrodt, E., and Rapp, U.R. (2007). Anticancer Res. *27* (6B), 3963–3971.

Metallo, C.M., Gameiro, P.A., Bell, E.L., Mattaini, K.R., Yang, J., Hiller, K., Jewell, C.M., Johnson, Z.R., Irvine, D.J., Guarente, L., et al. (2012). Nature *481*, 380–384.

Metellus, P., Colin, C., Taieb, D., Guedj, E., Nanni-Metellus, I., de Paula, A.M., Colavolpe, C., Fuentes, S., Dufour, H., Barrie, M., et al. (2011). J. Neurooncol. *105*, 591–600.

Mullen, A.R., Wheaton, W.W., Jin, E.S., Chen, P.H., Sullivan, L.B., Cheng, T., Yang, Y., Linehan, W.M., Chandel, N.S., and DeBerardinis, R.J. (2012). Nature *481*, 385–388.

Nieman, K.M., Kenny, H.A., Penicka, C.V., Ladanyi, A., Buell-Gutbrod, R., Zillhardt, M.R., Romero, I.L., Carey, M.S., Mills, G.B., Hotamisligil, G.S., et al. (2011). Nat. Med. *17*, 1498–1503.

Noushmehr, H., Weisenberger, D.J., Diefes, K., Phillips, H.S., Pujara, K., Berman, B.P., Pan, F., Pelloski, C.E., Sulman, E.P., Bhat, K.P., et al; Cancer Genome Atlas Research Network. (2010). Cancer Cell *17*, 510–522.

Ochoa, S. (1948). J. Biol. Chem. *174*, 133–157.

Ooi, A., Wong, J.C., Petillo, D., Roossien, D., Perrier-Trudova, V., Whitten, D., Min, B.W., Tan, M.H., Zhang, Z., Yang, X.J., et al. (2011). Cancer Cell *20*, 511–523.

Owen, M.R., Doran, E., and Halestrap, A.P. (2000). Biochem. J. *348*, 607–614.

Pansuriya, T.C., van Eijk, R., d'Adamo, P., van Ruler, M.A., Kuijjer, M.L., Oosting, J., Cleton-Jansen, A.M., van Oosterwijk, J.G., Verbeke, S.L., Meijer, D., et al. (2011). Nat. Genet. *43*, 1256–1261.

Papandreou, I., Cairns, R.A., Fontana, L., Lim, A.L., and Denko, N.C. (2006). Cell Metab. *3*, 187–197.

Parsons, D.W., Jones, S., Zhang, X., Lin, J.C., Leary, R.J., Angenendt, P., Mankoo, P., Carter, H., Siu, I.M., Gallia, G.L., et al. (2008). Science *321*, 1807–1812.

Pisano, M., Cocco, P., Cherchi, R., Onnis, R., and Cherchi, P. (1991). Tumori *77*, 12–15.

Pope, W.B., Prins, R.M., Albert Thomas, M., Nagarajan, R., Yen, K.E., Bittinger, M.A., Salamon, N., Chou, A.P., Yong, W.H., Soto, H., et al. (2012). J. Neurooncol. *107*, 197–205.

Possemato, R., Marks, K.M., Shaul, Y.D., Pacold, M.E., Kim, D., Birsoy, K., Sethumadhavan, S., Woo, H.K., Jang, H.G., Jha, A.K., et al. (2011). Nature *476*, 346–350.

Ramanathan, A., and Schreiber, S.L. (2009). Proc. Natl. Acad. Sci. USA *106*, 22229–22232.

Rathmell, J.C., Vander Heiden, M.G., Harris, M.H., Frauwirth, K.A., and Thompson, C.B. (2000). Mol. Cell *6*, 683–692.

Rathmell, J.C., Fox, C.J., Plas, D.R., Hammerman, P.S., Cinalli, R.M., and Thompson, C.B. (2003). Mol. Cell. Biol. *23*, 7315–7328.

Rempel, A., Mathupala, S.P., Griffin, C.A., Hawkins, A.L., and Pedersen, P.L. (1996). Cancer Res. *56*, 2468–2471.

Rydström, J. (2006). Biochim. Biophys. Acta *1757*, 721–726.

Schieke, S.M., Phillips, D., McCoy, J.P., Jr., Aponte, A.M., Shen, R.F., Balaban, R.S., and Finkel, T. (2006). J. Biol. Chem. *281*, 27643–27652.

Scholnick, P., Lang, D., and Racker, E. (1973). J. Biol. Chem. *248*, 5175.

Selak, M.A., Armour, S.M., MacKenzie, E.D., Boulahbel, H., Watson, D.G., Mansfield, K.D., Pan, Y., Simon, M.C., Thompson, C.B., and Gottlieb, E. (2005). Cancer Cell *7*, 77–85.

Shah, U.S., Dhir, R., Gollin, S.M., Chandran, U.R., Lewis, D., Acquafondata, M., and Pflug, B.R. (2006). Hum. Pathol. *37*, 401–409.

Shen, C., Beroukhim, R., Schumacher, S.E., Zhou, J., Chang, M., Signoretti, S., and Kaelin, W.G., Jr. (2011). Cancer Discov *1*, 222–235.

Shimada, N., Shinagawa, T., and Ishii, S. (2008). Genes Cells *13*, 245–254.

Siwko, S., and Mochly-Rosen, D. (2007). Int. J. Biochem. Cell Biol. *39*, 978–987.

Smith, E.H., Janknecht, R., and Maher, L.J., 3rd. (2007). Hum. Mol. Genet. *16*, 3136–3148.

Sonveaux, P., Végran, F., Schroeder, T., Wergin, M.C., Verrax, J., Rabbani, Z.N., De Saedeleer, C.J., Kennedy, K.M., Diepart, C., Jordan, B.F., et al. (2008). J. Clin. Invest. *118*, 3930–3942.

Spoden, G.A., Mazurek, S., Morandell, D., Bacher, N., Ausserlechner, M.J., Jansen-Dürr, P., Eigenbrodt, E., and Zwerschke, W. (2008). Int. J. Cancer *123*, 312–321.

Spoden, G.A., Morandell, D., Ehehalt, D., Fiedler, M., Jansen-Dürr, P., Hermann, M., and Zwerschke, W. (2009). J. Cell. Biochem. *107*, 293–302.

Sreekumar, A., Poisson, L.M., Rajendiran, T.M., Khan, A.P., Cao, Q., Yu, J., Laxman, B., Mehra, R., Lonigro, R.J., Li, Y., et al. (2009). Nature *457*, 910–914.

Srere, P.A. (1959). J. Biol. Chem. *234*, 2544–2547.

Stern, J.R., Ochoa, S., and Lynen, F. (1952). J. Biol. Chem. *198*, 313–321.

Steták, A., Veress, R., Ovádi, J., Csermely, P., Kéri, G., and Ullrich, A. (2007). Cancer Res. *67*, 1602–1608.

Stryer, L. (1995). Biochemistry, Fourth Edition (New York: W.H. Feeman and Company).

Tallal, L., Tan, C., Oettgen, H., Wollner, N., McCarthy, M., Helson, L., Burchenal, J., Karnofsky, D., and Murphy, M.L. (1970). Cancer *25*, 306–320.

Turcan, S., Rohle, D., Goenka, A., Walsh, L.A., Fang, F., Yilmaz, E., Campos, C., Fabius, A.W., Lu, C., Ward, P.S., et al. (2012). Nature. Published online February 15, 2012. 10.1038/nature10866.

Vander Heiden, M.G., Cantley, L.C., and Thompson, C.B. (2009). Science *324*, 1029–1033.

Vander Heiden, M.G., Locasale, J.W., Swanson, K.D., Sharfi, H., Heffron, G.J., Amador-Noguez, D., Christofk, H.R., Wagner, G., Rabinowitz, J.D., Asara, J.M., and Cantley, L.C. (2010). Science *329*, 1492–1499.

Wakil, S.J., Porter, J.W., and Gibson, D.M. (1957). Biochim. Biophys. Acta *24*, 453–461.

Wang, J.B., Erickson, J.W., Fuji, R., Ramachandran, S., Gao, P., Dinavahi, R., Wilson, K.F., Ambrosio, A.L., Dias, S.M., Dang, C.V., and Cerione, R.A. (2010). Cancer Cell *18*, 207–219.

Ward, P.S., Patel, J., Wise, D.R., Abdel-Wahab, O., Bennett, B.D., Coller, H.A., Cross, J.R., Fantin, V.R., Hedvat, C.V., Perl, A.E., et al. (2010). Cancer Cell *17*, 225–234.

Ward, P.S., Cross, J.R., Lu, C., Weigert, O., Abel-Wahab, O., Levine, R.L., Weinstock, D.M., Sharp, K.A., and Thompson, C.B. (2011). Oncogene. Published online September 26, 2011. 10.1038/onc.2011.416.

Watanabe, T., Nobusawa, S., Kleihues, P., and Ohgaki, H. (2009). Am. J. Pathol. *174*, 1149–1153.

Weinberg, F., Hamanaka, R., Wheaton, W.W., Weinberg, S., Joseph, J., Lopez, M., Kalyanaraman, B., Mutlu, G.M., Budinger, G.R., and Chandel, N.S. (2010). Proc. Natl. Acad. Sci. USA *107*, 8788–8793.

Wellen, K.E., Lu, C., Mancuso, A., Lemons, J.M., Ryczko, M., Dennis, J.W., Rabinowitz, J.D., Coller, H.A., and Thompson, C.B. (2010). Genes Dev. *24*, 2784–2799.

Williams, J.M., Chen, G.C., Zhu, L., and Rest, R.F. (1998). Mol. Microbiol. *27*, 171–186.

Williams, S.C., Karajannis, M.A., Chiriboga, L., Golfinos, J.G., von Deimling, A., and Zagzag, D. (2011). Acta Neuropathol. *121*, 279–281.

Wise, D.R., DeBerardinis, R.J., Mancuso, A., Sayed, N., Zhang, X.Y., Pfeiffer, H.K., Nissim, I., Daikhin, E., Yudkoff, M., McMahon, S.B., and Thompson, C.B. (2008). Proc. Natl. Acad. Sci. USA *105*, 18782–18787.

Wise, D.R., Ward, P.S., Shay, J.E., Cross, J.R., Gruber, J.J., Sachdeva, U.M., Platt, J.M., DeMatteo, R.G., Simon, M.C., and Thompson, C.B. (2011). Proc. Natl. Acad. Sci. USA *108*, 19611–19616.

Wu, X., Zhou, Y., Zhang, K., Liu, Q., and Guo, D. (2008). FEBS Lett. *582*, 2155–2160.

Xu, W., Yang, H., Liu, Y., Yang, Y., Wang, P., Kim, S.H., Ito, S., Yang, C., Wang, P., Xiao, M.T., et al. (2011). Cancer Cell *19*, 17–30.

Yan, H., Parsons, D.W., Jin, G., McLendon, R., Rasheed, B.A., Yuan, W., Kos, I., Batinic-Haberle, I., Jones, S., Riggins, G.J., et al. (2009). N. Engl. J. Med. *360*, 765–773.

Yang, W., Xia, Y., Ji, H., Zheng, Y., Liang, J., Huang, W., Gao, X., Aldape, K., and Lu, Z. (2011). Nature *480*, 118–122.

Yoo, H., Antoniewicz, M.R., Stephanopoulos, G., and Kelleher, J.K. (2008). J. Biol. Chem. *283*, 20621–20627.

Yuan, J., Bennett, B.D., and Rabinowitz, J.D. (2008). Nat. Protoc. *3*, 1328–1340.

Yun, J., Rago, C., Cheong, I., Pagliarini, R., Angenendt, P., Rajagopalan, H., Schmidt, K., Willson, J.K., Markowitz, S., Zhou, S., et al. (2009). Science *325*, 1555–1559.

Yuneva, M., Zamboni, N., Oefner, P., Sachidanandam, R., and Lazebnik, Y. (2007). J. Cell Biol. *178*, 93–105.

Zaugg, K., Yao, Y., Reilly, P.T., Kannan, K., Kiarash, R., Mason, J., Huang, P., Sawyer, S.K., Fuerth, B., Faubert, B., et al. (2011). Genes Dev. *25*, 1041–1051.

Zhang, W.C., Shyh-Chang, N., Yang, H., Rai, A., Umashankar, S., Ma, S., Soh, B.S., Sun, L.L., Tai, B.C., Nga, M.E., et al. (2012). Cell *148*, 259–272.

Zhao, F., Mancuso, A., Bui, T.V., Tong, X., Gruber, J.J., Swider, C.R., Sanchez, P.V., Lum, J.J., Sayed, N., Melo, J.V., et al. (2010). Oncogene *29*, 2962–2972.

Zhao, S., Lin, Y., Xu, W., Jiang, W., Zha, Z., Wang, P., Yu, W., Li, Z., Gong, L., Peng, Y., et al. (2009). Science *324*, 261–265.

Zscharnack, K., Kessler, R., Bleichert, F., Warnke, J.P., and Eschrich, K. (2009). Neuropathol. Appl. Neurobiol. *35*, 566–578.

Zwerschke, W., Mazurek, S., Massimi, P., Banks, L., Eigenbrodt, E., and Jansen-Dürr, P. (1999). Proc. Natl. Acad. Sci. USA *96*, 1291–1296.