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Novel Therapeutic Targets of Tumor Metabolism

Rigel J. Kishton[*,a] and **Jeffrey C. Rathmell**[*,†,‡,b]

[*]Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC 27710, USA

[†]Department of Immunology, Duke University Medical Center, Durham, NC 27710, USA

[‡]Duke Molecular Physiology Institute, Duke University Medical Center, Durham, NC 27710, USA

Abstract

The study of tumor metabolism has resulted in new understandings of how cancer cells modify metabolic pathways that control cellular energetics to allow increased proliferation and survival. Tumor cells have been shown to alter metabolic pathways involved in glucose, glutamine and mitochondrial metabolism to generate raw materials needed for rapid cellular proliferation, maintain favorable cellular redox environments, modify cellular epigenetics and even promote and maintain oncogenic transformation. As a consequence, there has been intense scientific and clinical interest in targeting metabolic alterations that are commonly adopted by tumor cells for therapeutic purposes. In this review, we describe common metabolic alterations seen in tumor cells and discuss how these alterations are being investigated as potential targets for pharmacological intervention in preclinical and clinical settings. We also discuss some of the challenges associated with using tumor metabolism as a therapeutic target in cancer therapy, along with potential avenues to overcome these challenges.

Keywords

Tumor metabolism; aerobic glycolysis; glutamine; IDH mutations; combination therapy

Therapeutic strategies for the treatment of cancer have undergone a revolution in recent years. In the past, frontline cancer therapy consisted largely of widely cytotoxic drugs that damaged both cancer cells and normal, healthy tissue. Over the past several decades, emphasis has been placed on identifying distinctive or preferential features of cancer cells to better target cancer yet spare normal cells. This has resulted in targeted therapies with higher treatment efficacy and improved patient outcomes. One recent focus in cancer research has been to exploit metabolic features of cancer cells that may separate them from normal tissue. The rationale behind this endeavor is that cancer cells, due to their rapid, sustained growth and proliferation and need to withstand hypoxia, must employ a metabolic program that

Address correspondence to: Jeffrey Rathmell, DUMC Box 3813, Duke University Medical Center, Durham, NC, 27710, Phone: 919-681-1084, Fax: 919-613-6606, jeff.rathmell@duke.edu.
[a]Doctoral candidate, MS
[b]Associate Professor, PhD

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deviates from the metabolic characteristics of normal, healthy tissue. The ability to target cancer metabolism may result in a therapeutic window where cancer cells are inhibited but normal healthy cells remain unaffected.

Metabolic Features of Cancer

Researchers and clinicians working in cancer related fields have come to understand that cancer is a heterogeneous disease. There is tremendous variation in biology between different types of cancer, different patients with the same type of cancer, and even between different cancer cells in the same tumor. Cancer metabolism also exhibits considerable heterogeneity, with tumors adopting various metabolic programs that best suit a particular microenvironment. There are, however, some commonalities in cancer metabolism that are generally applicable to a significant portion of tumors. Among these common features of cancer metabolism are alterations in glucose, glutamine and mitochondrial metabolism. These commons features may allow for the development of novel therapeutics to target a fundamental hallmark of cancer biology. Indeed, the fundamental nature of some metabolic pathways may provide a unified target to bypass and overcome the genetic heterogeneity of tumors.

Aerobic glycolysis in cancer

The metabolic needs of most normal differentiated cells are largely energetic and metabolism is oriented towards maximal ATP generation through oxidative phosphorylation. During this process, glucose is first converted through glycolysis to pyruvate in the cytosol of the cell then further metabolized in the mitochondria in the TCA cycle and electron transport to produce an electromotive force that generates large quantities of ATP. Otto Warburg first noted nearly 100 years ago, however, that cancer cells use an altered program of glucose metabolism, in which they, even under normoxic conditions, convert glucose-derived pyruvate to lactate, rather than oxidizing it in the mitochondria[1]. This metabolic program differed from conventional models at the time, where lactate was thought produced only in anaerobic conditions, and instead resembles the Crabtree effect, wherein respiration is suppressed the presence of oxygen if glucose levels are sufficiently high. Warburg termed this metabolic trait aerobic glycolysis and hypothesized it resulted from mitochondrial defects in cancer cells that rendered them unable to utilize a normal, oxidative metabolic program. While it has since been found that most cancer cells have intact mitochondria, it is certainly true that a number of oncogenic driver mutations shift cell metabolism away from oxidative phosphorylation towards glycolytic metabolism[2, 3]. The teleological reason for this metabolic reprogramming remains uncertain, but a strong consensus has emerged that glycolytic use of glucose allows cancer cells to generate biosynthetic intermediates that are necessary for cell growth and proliferation, while also avoiding the production of potentially harmful reactive oxygen species (ROS) that results from oxidative phosphorylation. This view is buttressed by findings of similar metabolisms in proliferative normal healthy cells, such as lymphocytes and endothelial cells in angiogenesis.

Since Warburg's description of aerobic glycolysis in cancer cells, key questions that have been studied are to what extent cancer cells require this metabolic program and can it be inhibited to eliminate cancer cells without excessive toxicity. There are a great number of metabolic pathways that utilize glucose or its derivatives towards the production of raw materials for biosynthesis, maintaining a favorable redox environment, and meeting the energy demands of cancer cells. Likewise amino acids can feed into a wide array of metabolic pathways. Given that core metabolic pathways are shared in nearly all cells, specificity for cancer cells and toxicity to normal cells are critical concerns. Also important is to what extent cancer cells can exert plasticity and respond to metabolic inhibition with compensatory metabolic reprogramming that may maintain cancer cell proliferation or viability.

Targeting Aerobic Glycolysis

There are numerous proteins that regulate the glycolytic pathway and have been proposed as potential drug targets. Here we will discuss the potential for targeting the early stages of glycolysis, where glucose is taken up into the cancer cell and phosphorylated to trap it in the cell, and the late stages of glycolysis, at the branch point where glucose derived pyruvate is either fluxed into the TCA cycle as acetyl-CoA, or converted to lactate for export (Figure1). The first two steps of glycolysis are the uptake of glucose into the cell by glucose transporters and subsequent phosphorylation by hexokinases. In numerous types of cancer, glucose transporters[4-6] and various isoforms of hexokinase are overexpressed[7], making them tempting targets for pharmacological inhibition. Indeed, the genetic deletion of Glut1 in a mouse model of B cell acute lymphoblastic leukemia (B-ALL) greatly slowed cell proliferation and lessened disease burden[8]. Similarly, the inhibition of glucose transporters has been explored in several cancer settings. For example, small molecule based inhibition of Glut1 was found to slow the growth of non-small cell lung cancer (NSCLC)[9]and have effects against renal cell carcinoma[3]. A number of drugs in the retroviral protease inhibitor class, commonly used to treat HIV infection, have been found to also possess the off-target effect of inhibiting glucose transporters, including Glut1 and Glut4[10]. Ritonavir, a drug in this class, has been shown to have anti-proliferative effects in a mouse model of multiple myeloma through the inhibition of glucose uptake into the cells[11]. When considering glucose transporters as a potential therapeutic target for human cancer patients, it must be noted that it is unclear what toxicities would occur with potent inhibition. For instance, Glut1 is heavily expressed at the blood brain barrier[12], and inhibition may result in neurological effects, as evidenced by patients with Glut1-deficiency Syndrome[13]. Nevertheless, Glut1 inhibitors with proven clinical track records, such as Ritonavir, show that a therapeutic window of partial inhibition of glucose uptake may be present.

Hexokinase may also provide a target in cancer metabolism through isoform selective inhibition. Several different types of cancer have been shown to overexpress Hexokinase II, an isoform not expressed in most normal tissue. Multiple groups have shown that the genetic deletion of Hexokinase II is beneficial, slowing cancer progression and reducing cancer cell survival in several different types of cancer, including lung, breast[14] and brain[15, 16]. Interestingly, while germline deletion of Hexokinase II is embryonic lethal in mice, whole body knockout in adult mice was reported well tolerated[14, 16], demonstrating that cancer

cells may selectively rely on this isoform that could allow therapeutic targeting of Hexokinase II in cancer. Small molecules that broadly inhibit hexokinase, such as 2-deoxyglucose (2-DG), have been shown to have activity against cancer *in vitro*[17-19] although *in vivo* efficacy of 2-DG as a single agent is modest[8, 20]. However, these compounds are not specific for a particular hexokinase isoform, and continued development of small molecules targeted at hexokinase isoforms overexpressed in cancer may provide improved specificity.

An important early step in glycolysis is the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate by 6-phosphofructo-1-kinase (PFK1). This is the first committed step in glycolysis, and the activity of PFK1 is elevated in many types of cancer, allowing for increased flux of glucose into the glycolytic pathway [21, 22]. The mechanism of increased PFK1 activity in cancer relies upon the generation of an allosteric activator of PFK1. Oncogenic signaling increases the expression of an isoform of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB) family of enzymes known as PFKFB3[23, 24]. Increased PFKFB3 expression results in the production of fructose-2,6,-bisphosphate, a potent allosteric activator of PFK1[25, 26]. Studies suggest that inhibition of PFKFB3 using genetic approaches[27] and small molecule inhibition[28] results in dramatically reduced glycolytic flux and slowed cancer cell growth. Early phase clinical trials are currently underway with small molecule PFKFB3 inhibitors [29].

Another key step in glucose metabolism is the branch point at which glycolysis-derived pyruvate can either be imported into the mitochondria to be oxidized in the TCA cycle, or converted to lactate in the cytosol. The pyruvate dehydrogenase (PDH) complex, which converts pyruvate to acetyl-CoA in the mitochondria, is responsible for regulating this key junction in pyruvate fate. An important regulator of PDH activity is pyruvate dehydrogenase kinase (PDHK). PDHK reduces the activity of PDH via inhibitory phosphorylation[30], resulting in decreased flux of pyruvate into the mitochondria, and increased production of lactate[31]. Several isoforms of PDHK have been shown to be overexpressed in various cancers [32-34], and play an important role in maintaining aerobic glycolysis in tumors. Numerous studies have shown that the inhibition of PDHK through RNAi or a small molecule inhibitor, dichloroacetate (DCA), caused cancer cell death *in vitro* and improved outcome in *in vivo* models of disease[35-37]. DCA was shown to alter the energetic balance of cancer cells, promoting the oxidation of glucose and consequent production of ROS[36-39]. DCA has been utilized clinically for the treatment of lactic acidosis[40], and several clinical trials have explored DCA as an anti-cancer treatment. In a small clinical trial, DCA treatment was associated with radiological regression of glioblastoma multiforme (GBM) in some patients, along with reduced proliferation and increased apoptosis of cancer cells[38]. Targeting PDHK with DCA or other novel small molecule inhibitors may be an effective strategy for the inhibition of aerobic glycolysis.

The lactate dehydrogenase (LDH) complex also plays a key role to regulate the fate of pyruvate in cancer. LDH is responsible for the conversion of pyruvate to lactate in the cytosol of the cell, and has increased expression and activity in a variety of cancer types[41, 42]. There are two isoforms of LDH that form tetramers of mixed composition[43] and increased presence of the LDHa isoform is often implicated in contributing to aerobic

glycolysis in cancer cells[41, 42, 44]. Of the isoforms of LDH, LDHa has the highest affinity for pyruvate, along with the highest Vmax for enzymatic activity[45]. Thus, LDHa is able to rapidly convert pyruvate into lactate, completing aerobic glycolysis. There are several hypothesized reasons for cancer cells to overexpress LDHa and to convert pyruvate to lactate. The reaction catalyzed by LDHa results in the production of NAD+, which is critical for maintaining the activity of other proteins in the glycolytic pathway such as GAPDH[45, 46]. Also, studies have shown that LDHa activity is critical for keeping a favorable redox environment in cancer cells[47]. Several research groups have shown that the inhibition of LDHa by small molecule inhibitors or genetic approaches results in slowed cancer cell growth and increased cell death in a variety of types of cancer settings, including hepatocellular carcinoma and breast cancer[47-49]. There have been several early stage clinical trials to evaluate the efficacy of a non-specific inhibitor of LDH, with mixed results observed[50, 51]. The pre-clinical development of inhibitors that have more specificity for LDHa is currently ongoing [52, 53].

Glutamine metabolism in cancer

In addition to altered glucose metabolism cancer cells can have increased usage of and reliance on glutamine for cell growth and survival. Dramatically increased usage of glutamine is a metabolic phenotype often associated with oncogenic Myc signaling[54], but is also found in tumors with other driver mutations such as oncogenic KRAS[55]. Glutamine serves several purposes to tumor cells (Figure 2). The first step in glutamine metabolism is its import into the cell via glutamine transporters. There are several known transporters that are capable of taking up glutamine, with SLC1A5 (ASCT2) and LAT1 being commonly upregulated in malignancies[56-58]. Once cytosolic, glutamine can be used as a substrate for the de novo synthesis of proteins, purines and pyrimidines[59], or can be converted to glutamate by enzymes called glutaminases (GLS). After conversion, glutamine derived glutamate can then be utilized by cancer cells for a variety of important purposes[60, 61]. One of these is the generation of non-essential amino acids for growth and proliferation through transamination of glutamate. Glutamate also plays an important role as a carbon donor in cancer cell TCA flux. Glutamate can be converted to α-ketoglutarate by glutamate dehydrogenase (GDH) and then fluxed into the TCA cycle where it can be used to support oxidative phosphorylation, the production of lipids, or to replenish key intermediates such as oxaloacetate[62, 63]. Glutamate can also be used to produce reducing agents for the cell, either being converted into glutathione[64], or generating NADPH through malic enzyme[65].

Targeting glutamine metabolism

The increased reliance by some cancers on glutamine metabolism provides several targets for therapeutic intervention. Several groups have explored the inhibition of glutamine transporters to limit glutamine uptake into cancer cells. LAT1 is inhibited by the small molecule inhibitor 2-amino-(2,2,1)-heptane-2-carboxylic acid (BCH), and the treatment of cancer cells *in vitro* and *in vivo* with BCH or genetic knockdown of LAT1 has been shown to slow proliferation and tumor growth[66, 67]. The inhibition of ASCT2 by RNAi or the small molecule L-γ-glutamyl-p-nitroanilide (GPNA) has also been shown to decrease pro-

growth mTOR signaling and to induce autophagy in cancer cells[68]. Another study found that ASCT2 inhibition caused reduced growth and viability in several subtypes of lung cancer cells, effects that were mediated through a reduction in mTOR pathway activity [69]. Another potential therapeutic target in glutamine metabolism is GLS. A number of small molecule inhibitors of GLS have been developed, among them bis-2-(5-phenylacetamido-1,2,4-thiodiazol-2-yl)ethyl sulfide (BPTES). BPTES has been shown to successfully inhibit GLS activity in several cancer settings, resulting in slowed growth and cell death [70, 71]. There has also been interest in limiting the process of glutamine entering the TCA cycle as α-ketoglutarate by inhibiting GDH. Currently, there are no small molecule inhibitors that are specific for GDH[61]. However, non-specific inhibitors of GDH such as epigallocatechin gallate (EGCG) and aminooxyacetate (AOA) have been successfully demonstrated to be toxic to cancer cells *in vitro*, and to slow the growth of xenografted tumors[72, 73]. The development of more specific inhibitors of GDH may allow for more efficacious targeting of glutamine flux into the TCA cycle.

Alterations to the TCA cycle in cancer metabolism

In addition to utilizing aerobic glycolysis and glutamine metabolism for proliferation and survival, it has become clear in recent years that some cancer cells extensively also alter normal TCA cycle metabolism towards these ends (Figure 3). Typically thought of as acting in support of oxidative mitochondrial metabolism, the TCA cycle can also be adapted to produce cell building blocks for proliferation. As one example, citrate produced from acetyl CoA in the TCA cycle can be exported from the mitochondria and converted into raw material for the synthesis of fatty acids that are needed for cell proliferation. The TCA cycle flow can be reversed in reductive carboxylation so that α-ketoglutarate is converted to isocitrate then citrate for lipid synthesis[74, 75]. Also, interestingly, mutations that contribute to oncogenesis and the maintenance and progression of established tumors have been identified in several TCA enzymes. To date, several mutations have been identified in TCA cycle enzymes, including succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH). SDH and FH have come to be thought of as tumor suppressors, as mutations in either enzyme has been shown to cause sarcomas, renal cell carcinoma and other rare types of cancer[76-78]. IDH mutations are found in gliomas[79, 80] and acute myeloid leukemias[81], and evidence implicates them in other cancer settings[82-85]. These mutations result in a gain of function, allowing IDH to begin producing a new “oncometabolite” called (R)-2-hydroxyglutarate (2HG)[86, 87]. 2HG itself has been termed an oncometabolite and has the capacity to transform immortalized cells *in vitro*[88, 89], through mechanisms that remain somewhat unclear. Numerous studies provide evidence that increased 2HG in cells harboring IDH mutations can inhibit demethylases, leading to hypermethylated DNA and retention of a stem cell-like phenotype[89-92].

Targeting the TCA cycle in cancer metabolism

Small molecule based targeting of abnormalities in the TCA cycle has become one of the greatest successes to date in therapeutically attacking cancer metabolism. While success targeting mutant FH and SDH with small molecule inhibitors has been limited because these are loss of function mutations, novel compounds that inhibit the gain-of-function activity of

mutant IDH have recently been shown to have success in preclinical and clinical settings. In preclinical studies, small molecule inhibition of mutant IDH has been shown to dramatically reduce the production of 2HG and cause cancerous cells to differentiate towards a more normal phenotype[93, 94]. Early phase clinical trials have begun with a small molecule inhibitor of mutant IDH2, AG-221.

Challenges of targeting cancer metabolism

While there are numerous potential therapeutic targets in cancer metabolism, there are profound challenges associated with utilizing metabolic inhibition as a clinical strategy. First among these challenges is the fact that it is difficult to achieve a therapeutic window in cancer metabolism, as many normal cells, especially rapidly proliferating cells of the immune system, also utilize metabolic programs similar to those utilized by cancer and toxicity could be significant by targeting some metabolic pathways. For example, effector subtypes of T cells and antibody producing B cells also rely on aerobic glycolysis[95-97] and glutamine metabolism[98, 99] to maintain immune function. Metabolic inhibition of immune cells could potentially reduce their ability to fight cancer, and further, leave patients more vulnerable to opportunistic infections. Another challenge in targeting cancer metabolism is the metabolic flexibility that many tumor cells exhibit. Except for cases where there are actual mutations in metabolic genes, cancer cells often have a remarkable ability to shift fuel sources when deprived of favored metabolic pathways[8, 73, 100, 101]. This metabolic flexibility may limit the efficacy of targeting a single pathway for therapeutic purposes.

Combination therapy as a solution

One potential way to overcome the challenges posed to successfully utilize cancer metabolism as a therapeutic target is to utilize combination therapy. There are several potential ways in which metabolic combination therapy could be used against cancer cells. Inhibiting a primary metabolic pathway, followed by the subsequent inhibition of alternate metabolic pathways used by tumor cells might be one strategy. Additionally, many groups have shown that the partial inhibition of metabolic pathways utilized by cancer cells can dramatically sensitize the cancer cells to more traditional chemotherapeutic drugs or targeted therapies[8, 102-105]. This adjuvant metabolic sensitization may allow the use of far lower doses of both metabolic inhibitors and chemotherapeutic agents to achieve greater efficacy against tumor cells and reduced off-target effects.

Conclusions

The therapeutic potential of targeting the alterations of cellular metabolism in cancer has existed since the description of aerobic glycolysis by Otto Warburg. In the decades since Warburg's observation, much progress has been made in understanding exactly how many types of cancer alter cellular energetic pathways and how these alterations may be used to design novel therapeutic strategies to combat the disease. It is clear from recent research that there are a number of potential pathways and targets that may be beneficial targets for cancer therapy. In this review, we have described potential targets in the metabolic pathways

that regulate glycolysis, glutamine metabolism, and the TCA cycle. It is likely that research in the coming years will identify more potential targets for therapeutic intervention. While there are numerous challenges associated with targeting cancer metabolism, among them off-target effects of metabolic inhibitors and the suppression of immune cells, strategies such as using metabolic inhibitors in combination therapy may allow for more effective clinical use.

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Glossary

2-DG	2-deoxyglucose
2HG	2-hydroxyglutarate
AOA	aminoxyacetate
ATP	adenosine triphosphate
B-ALL	B cell acute lymphoblastic leukemia
BCH	2-amino-(2,2,1)-heptane-2-carboxylic acid
BPTES	bis-2-(5-phenylacetamido-1,2,4-thiodiazol-2-yl)ethyl sulfide
DCA	dichloroacetate
EGCG	epigallocatechin gallate
FH	fumarate hydratase
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
GBM	glioblastoma multiforme
GDH	glutamate dehydrogenase
GLS	glutaminase
GPNA	L-γ-glutamyl-p-nitroanilide
IDH	isocitrate dehydrogenase
LAT1	large neutral amino acid transporter
LDH	lactate dehydrogenase
mTOR	mechanistic target of rapamycin
NAD+	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NSCLC	non-small cell lung cancer
PDH	pyruvate dehydrogenase
PDHK	pyruvate dehydrogenase kinase
PFK1	phosphofructokinase 1
PFKFB	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase
RNAi	RNA interference
ROS	reactive oxygen species
SDH	succinate dehydrogenase
SLC1A5 (ASCT2)	neutral amino acid transporter B(0)/ASC amino acid transporter 2
TCA cycle	tricarboxylic acid cycle

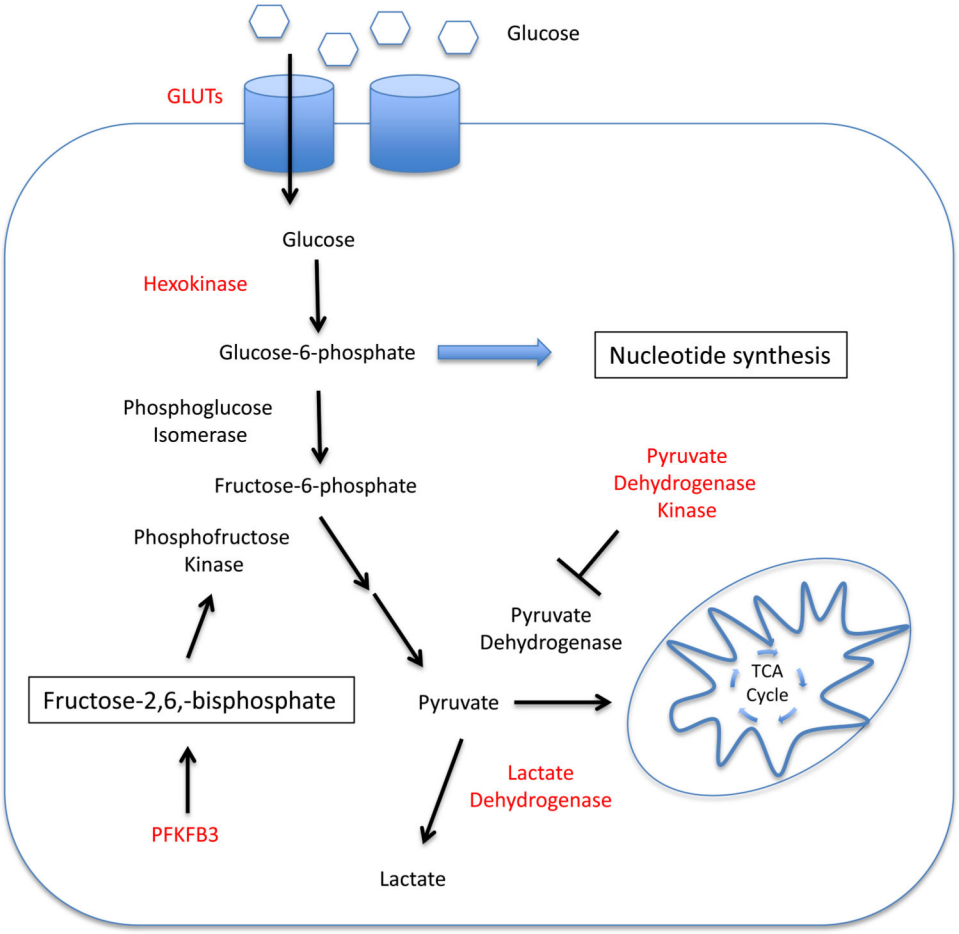

Figure 1. Tumor cell glucose metabolism as a therapeutic target

The alterations to normal glucose metabolism that are exhibited in cancer cells may provide targets for therapeutic intervention. Inhibiting aerobic glycolysis in cancer cells causes slowed cell proliferation and increased cell death. Enzymes that are currently being targeted with small molecule inhibitors are indicated in red.

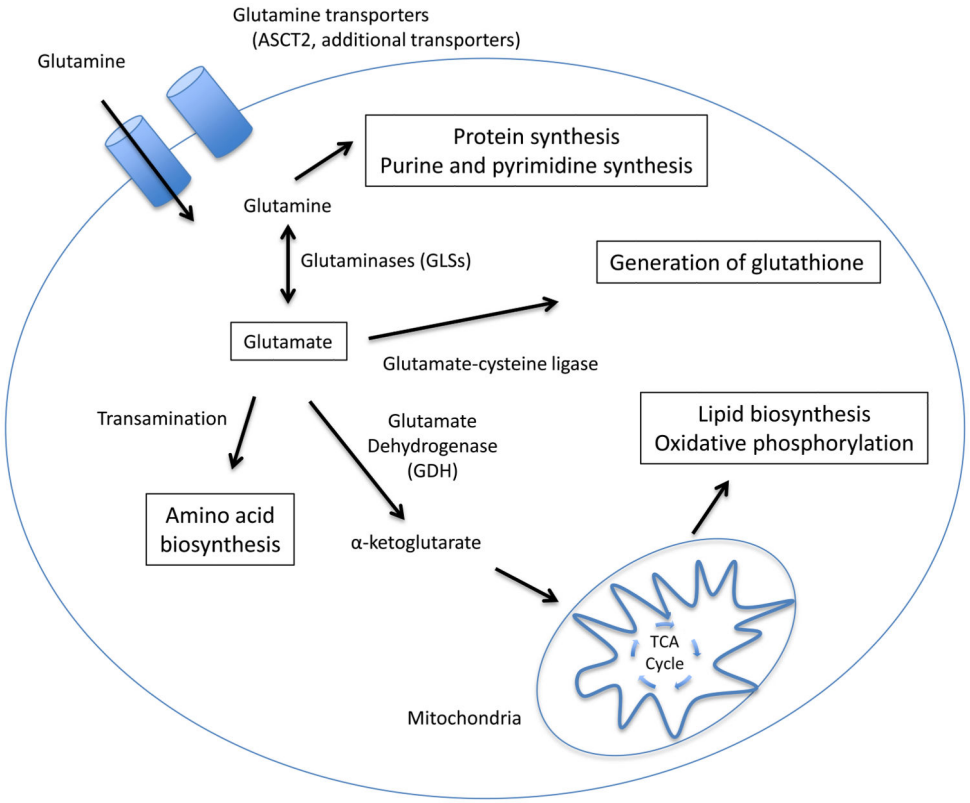

Figure 2. Glutamine is used for a number of anabolic processes by cancer cells

Glutamine is transported inside tumor cells by glutamine transporters such as ASCT2. Once inside the cell, it can be used directly as a substrate for protein and nucleotide synthesis, or converted to glutamate by glutaminases (GLS). Glutamine-derived glutamate has a number of uses for cancer cells. It may be used to generate amino acids via transamination, or used in the generation of reducing equivalents such as glutathione. Additionally, glutamate may be converted to α-ketoglutarate by glutamate dehydrogenase (GDH) and fluxed into the mitochondrial TCA cycle where it can be used to support oxidative phosphorylation, or used to generate lipids.

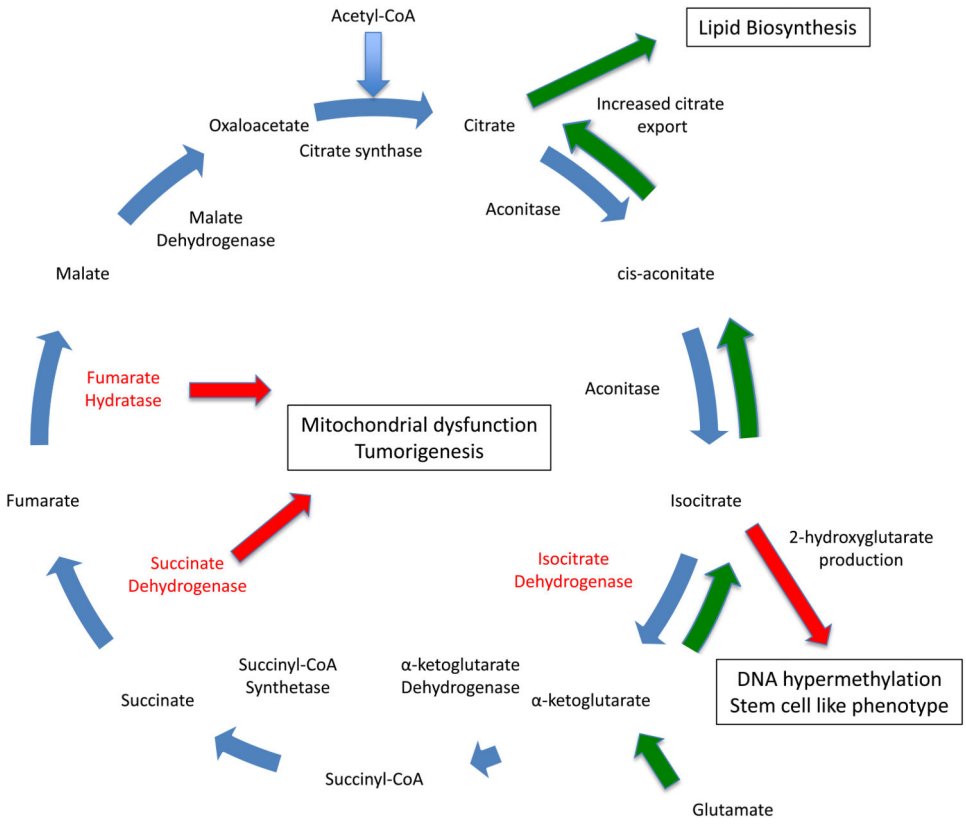

Figure 3. Cancer cells alter the TCA cycle to support proliferation and oncogenic transformation

Tumor cells often significantly alter the flow of TCA cycle intermediates (common alterations indicated with green arrows) to increase the generation of substrates useful for cell growth and proliferation. One common alteration in TCA cycle flux is the increased export of citrate from the TCA cycle to support de novo lipid synthesis for proliferation. Along with simply increasing the amount of citrate that is exported, some cancer cells also utilize glutamine-derived glutamate to generate citrate. In this process, glutamate is converted to α-ketoglutarate and fluxed into the TCA cycle. The TCA cycle flow is then reversed, with α-ketoglutarate being converted into isocitrate and eventually citrate to yield even more substrate for lipid synthesis. Tumor cells also are known to have mutations in key TCA cycle enzymes (enzymes known to be mutated indicated in red). Isocitrate dehydrogenase mutations can result in the generation of the “oncometabolite” 2-hydroxyglutarate, which contributes the a stem cell like phenotype in tumor cells. Additional mutations have been identified in fumarate hydratase and succinate dehydrogenase. These mutations result in mitochondrial dysfunction and contribute to oncogenic transformation.