Dysregulated metabolism contributes to oncogenesis

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Abstract

Cancer is a disease characterized by unrestrained cellular proliferation. In order to sustain growth, cancer cells undergo a complex metabolic rearrangement characterized by changes in metabolic pathways involved in energy production and biosynthetic processes. The relevance of the metabolic transformation of cancer cells has been recently included in the updated version of the review "Hallmarks of Cancer", where the dysregulation of cellular metabolism was included as an emerging hallmark. While several lines of evidence suggest that metabolic rewiring is orchestrated by the concerted action of oncogenes and tumor suppressor genes, in some circumstances altered metabolism can play a primary role in oncogenesis. Recently, mutations of cytosolic and mitochondrial enzymes involved in key metabolic pathways have been associated with hereditary and sporadic forms of cancer. Together, these results suggest that aberrant metabolism, once seen just as an epiphenomenon of oncogenic reprogramming, plays a key role in oncogenesis with the power to control both genetic and epigenetic events in cells. In this review, we discuss the relationship between metabolism and cancer, as part of a larger effort to identify a broad-spectrum of therapeutic approaches. We focus on major alterations in nutrient metabolism and the emerging link between metabolism and epigenetics. Finally, we discuss potential strategies to manipulate metabolism in cancer and tradeoffs that should be considered. More research on the suite of metabolic alterations in cancer holds the potential to discover novel approaches to treat it.

Keywords

Cancer metabolism; mitochondria; Warburg; host metabolism; cancer therapy

1. Introduction

A non-profit organization called Getting to Know Cancer launched an initiative entitled "The Halifax Project" in 2011, which was charged with identifying synergistic molecular targets and/or small molecules for each of the areas that are widely considered to be hallmarks of cancer [1]. The rationale for this approach is based on the idea that cancers harbor significant genetic heterogeneity [2], which is often not addressed with monotherapeutic approaches. While efforts have been made to combine therapies to overcome resistance, rising drug costs, significant levels of toxicity, and a lack of overall success have stymied efforts to effectively treat cancer with multi-drug combinations [3].

Thus, the first aim of the Halifax Project was to produce a series of reviews, including this review on cancer metabolism, to broadly assess current knowledge on the biology of cancer. The overall goal of the Halifax Project is to identify biological targets and prospective lead compounds that could potentially be used to reach each prioritized area, and synergistically target multiple hallmarks of cancer. By building this rationale into the approach *a priori*, the problem of heterogeneity might be overcome. In theory, multiple low toxicity approaches could be experimentally combined, which then might lead to synergism within a given hallmark, such as cancer metabolism. Future studies will build upon these findings and test these hypotheses, as well as integrate these concepts into the approaches recommended in other hallmark areas in this special issue.

In this review, we first discuss the relationships between metabolism and cancer. We focus on major alterations in nutrient metabolism, as well as the emerging links between metabolism and epigenetics. Next, we discuss potential therapeutic strategies that could be used to manipulate metabolism in cancer cells or to manipulate host metabolism thereby influencing cancer metabolism. Finally, we describe tradeoffs that should be considered when leveraging these approaches. Together, this information will be the basis of significant future research to fully realize the potential of targeting metabolism in cancer.

2. Classic Metabolic Derangements

The first realization that metabolism is altered in cancer can trace its roots to the work of Otto Warburg. During the 1920s, Warburg found that unlike most normal tissues, cancer tissues fermented glucose to lactate at high rates regardless of the presence of oxygen [4, 5]. This was in contrast to the results that Pasteur had obtained previously studying fermentation in yeast, whereby O_2 was found to inhibit fermentation [6, 7]. To study the metabolism of cancer *in vivo,* Warburg used Jensen sarcoma cells to form tumors within the abdomens of rats. By comparing arterial glucose and lactate concentrations to venous glucose and lactate concentrations, Warburg was able to infer the glucose uptake and lactate excretion by the tumor. Whereas normal tissues took up 2–18% of arterial glucose, tumors consumed 47–70%. Lactate was not significantly changed in blood after perfusion of normal tissues, but by Warburg's calculations, tumors converted 66% of their consumed glucose into lactate. Thus, Warburg surmised that tumors take up much more glucose than normal tissues and convert a much larger percentage of it to lactate [4].

Warburg's work on respiration and fermentation in cancer cells ultimately led him to propose that "the respiration of all cancer cells is damaged" [8]. In fact, he reasoned that known carcinogens, such as arsenic and hydrogen sulfide, likely worked by inhibiting respiration. He suggested that the primary oncogenic insult was an inability of cells to oxidize glucose carbons, and that X-rays were carcinogenic mainly due to their effect on mitochondria [8], which by this time had been shown to be the respiratory center of cells.

The exact molecular mechanisms leading to the Warburg effect and to altered metabolism in cancer remain a major unsolved question; for a review, see [9]. Subsequent studies have shown that while changes in mitochondrial respiration are sometimes seen in cancer cells, these alterations are not likely the driving lesion for most cancer cells. For example, Warburg's follow-up work suggested that oxidative respiration was important in malignant tumors, and reported that placing rats in 5% O_2 for 40 hours resulted in the death of most cancer cells, suggesting that oxygen was needed for viability of those cancer cells [4]. Similarly, the work of his contemporaries showed that oxygen consumption is intact in many cancers, thereby decoupling the Warburg effect from defective oxygen consumption [10, 11]. However, oxygen consumption cannot be a direct measurement of intact respiration, because mitochondrial coupling/uncoupling influences the efficiency of oxygen consumed to ATP produced. Nevertheless, many cancer cells display increased glucose uptake and elevated lactate production, irrespective of oxygen availability – also called "aerobic glycolysis" or the Warburg effect [12], and this observation remains a hallmark of altered metabolism in cancer cells.

3. Emerging Metabolic Derangements

While the mechanisms leading to the Warburg effect are under intense investigation, the general consensus of the field is that dysregulated metabolism and altered mitochondrial structure-function [13] is consistently found in several cancer cell types. These changes may occur before, as a result of, or in combination with, the genetic changes driving cancer, including oncogene expression or tumor suppressor loss; for recent comprehensive reviews on these concepts, see [14, 15]. For example, one well-studied link between oncogenesis and glucose metabolism is the phosphoinositide 3-kinase (PI3K) signaling pathway. Activating mutations in PI3K or overexpression of the AKT oncogenes, which lie downstream of PI3K, can induce high rates of aerobic glycolysis in non-transformed cells. This occurs in part by increasing expression and localization of the high-affinity glucose transporter, GLUT1, on the plasma membrane [16, 17]. In addition, activation of the PI3K pathway can accelerate flux through glycolysis by increasing the activity of hexokinase-2, phosphfructokinase-1 (PFK1), or phosphofructokinase-2 (PFK-2) [18–120]. The tumor suppressor p53, which has a well described role in DNA damage sensing, cell cycle control, and control of apoptosis, is also able to oppose the Warburg effect by stimulating respiration and reducing glycolytic flux [21–23]. Thus, loss of p53 in cancer cells is another event that can impact glucose metabolism in cancer cells.

Given the inextricable relationship between oncogenes, tumor suppressors, and the regulation of glycolysis, metabolic alterations including the Warburg effect could provide a selective advantage to rapidly proliferating cells [24]. Although fermentation produces almost 20 times less adenosine 5′-triphosphate (ATP) per glucose molecule than oxidative glucose metabolism, it has been suggested that ATP is never limiting in dividing cells [24, 25]. Instead, proliferating cells require macromolecular precursors and reducing power in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to synthesize new biomass. Therefore, one possible advantage of the Warburg effect is that high flux through glycolysis allows for more efficient use of glycolytic intermediates for NADPH production and biosynthetic pathways including lipid synthesis, nucleotide synthesis, and amino acid synthesis, which would be permissive for rapid cellular proliferation.

Although glucose metabolism is important for proliferating cells, it has recently been appreciated that other nutrient sources contribute as well. TCA cycle intermediates are required for biosynthetic processes, including the generation of citrate for lipid synthesis and aspartate for nucleotide synthesis. When these intermediates are removed from the TCA cycle, they must be replenished in a process known as anaplerosis. Glutamine has been shown to be a key contributor to anaplerotic flux in many cancer cells [26, 27]. Glutamine carbon entry into the TCA cycle supports both ATP generation and biosynthesis, and a wide variety of cancer cell types are sensitive to glutamine withdrawal [28, 29]. More recent work has suggested catabolism of extracellular protein can also contribute carbon to the TCA cycle [30], and lipids are scavenged to support proliferation of some cancer cells [31].

Collectively, it is becoming apparent that derangements to metabolism that enable the generation of biosynthetic precursors are a key feature in cancer initiation, development, and/or growth. Here, we discuss how alterations in key metabolic pathways contribute to

biomass generation (Table 1). We discuss some prioritized targets within these pathways, their therapeutic potential, and strategies to manipulate metabolism for the prevention or treatment of cancer.

4. Glucose Metabolism

4.1 Hexokinase 2 (HK2)

Regulation of glycolysis is exerted by the three important kinases that catalyze discrete phosphorylation reactions (Figure 1). Hexokinases are a family of enzymes that catalyze the first phosphorylation of glucose to glucose-6-phosphate. Early work on a highly malignant AS-30D hepatoma cell line showed that one isozyme of hexokinase was uniquely bound to the outer mitochondrial membrane [32, 33], which was later identified as Hexokinase 2 (HK2) [34].

In a series of experiments in this system, removal of malignant tumor mitochondria containing bound hexokinase from the cytoplasm by centrifugation markedly decreased the rate of glycolysis. Then, when tumor mitochondria containing bound hexokinase were added back to the tumor cytosol, the original glycolytic capacity of the cytoplasm was restored. Finally, when solubilized hexokinase alone was added to the liver cytosol, it markedly enhanced the glycolytic rate [34]. Therefore, HK2 could be a major contributor to high glycolysis and lactate production even in the presence of oxygen. One mechanism by which HK2 binds to mitochondria is via the outer membrane protein known as the voltage-dependent anion channel VDAC [35]. This binding interaction facilitates the immortalization of cancer cells [36, 37].

To identify compounds that could selectively inhibit the two main energy-producing pathways (glycolysis and mitochondrial oxidative phosphorylation), a limited screen was performed in cancer cells. The small molecule 3-bromopyruvate (3BP) was identified as an inhibitor of glycolysis and also as an inhibitor of mitochondrial energy production, which could be explained by inhibiting HK2 [38]. Follow-up work showed that 3BP has potent anticancer activity and has the capacity to eradicate cancers in different animal models [39]. Furthermore, 3BP was recently tested in a single human case study and showed promising results [40, 41]. Future work will be directed at the role of other HK isoforms in cancer, as well as the specificity of 3BP towards HK2. However, these early studies are one proof-of-principle that targeting energy metabolism of cancer cells can be an effective therapeutic approach.

4.2 6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 (PFKFB3)

Another gluco-regulatory kinase is phosphofructokinase (PFK), which catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate (Figure 1). PFK is regulated by several metabolites, including inhibition by high concentrations of ATP and activation by fructose 2,6-bisphosphate. Early studies indicated that phosphofructokinase activity correlated with the growth rate of Morris hepatomas transplanted in rats and also correlated with lactate production by slices of those tissues [42]. Those observations suggested that inhibition of phosphofructokinase activity represented a logical target for inhibition of malignant tumor growth. With the discovery that fructose 2,6-bisphosphate is

an activator of phosphofructokinase 1 [43], the enzyme activity catalyzing the formation of fructose 2,6-bisphosphate became an alternative target for the inhibition of glycolysis and cancer growth. Steady state levels of fructose 2,6-bisphosphate are regulated by bifunctional enzymes that have both phosphofructo-2-kinase and fructose-2,6-bisphosphatase (PFKFBs). Of these enzymes, PFKFB3 has the highest phosphofructo-2-kinase activity [44]. Interestingly, PFKFB3 expression, but not other PFKFBs, is markedly elevated in multiple aggressive primary cancers, including colon, breast, ovarian and thyroid carcinomas [45].

The role of PFKFB3 in the regulation of glucose metabolism in cancer cells has been reviewed previously [46]. An early indication that PFKFB3 might be a regulatory enzyme was the identification of multiple copies of the AUUUA instability motif in its 3′ untranslated region [44]. Expression of the PFKFB3 gene is induced by hypoxia through hypoxia-inducible factor-1 [47]. Low pH, a common feature in malignant tumors, is another factor that results in upregulation of PFKFB3. This may be mediated through the metabolic stress sensor AMP-activated protein kinase (AMPK) resulting in an increase in serine phosphorylation [48]. In breast cancer cells, synthetic progestins activate PFKFB3 isoenzyme phosphorylation and a long-term sustained action due to increased PFKFB3 protein levels. An immediate early response occurs through the ERK/RSK pathway leading to phosphorylation on S461 followed by activation of transcription via *cis*-acting sequences on the PFKFB3 promoter [49]. In myeloproliferative neoplasms, PFKFB3 expression was upregulated via active JAK2 and STAT5 [50], suggesting that specific inhibitors of PFKFB3 might inhibit JAK2/STAT5-dependent malignancies.

Levels of PFKFB3 are regulated by ubiquitinylation during the cell cycle. PFKFB3 accumulates in mid to late G1 and breakdown in S phase occurs specifically via a distinct S273 residue within the conserved recognition site for SCF-beta-TrCP [51]. Others have noted that the activity of PFKFB3 is short lasting, coinciding with a peak in glycolysis in mid to late G1 in contrast to glutamine metabolism, which remains high throughout S phase [52]. Glycolysis is characteristically associated with cytosolic fractions of cells but PFKFB3 has a nuclear localization signal. Furthermore, nuclear localization of PFKFB3 was associated with increased proliferation by increased expression of cyclin-dependent kinases and decreased expression of the cell cycle inhibitor p27 [53]. This brings to mind the more recent observation with the pyruvate kinase isoenzyme PKM2 (described below) that has been shown to have potential transcription regulatory action in addition to its role in glycolysis [54]. Therefore, a dual function may be associated with some regulatory enzymes involved in glycolysis.

Molecular modeling studies were the basis for the identification of the first published report of a small molecule inhibitor of PFKFB3 [55]. This molecule, 3-(3-pyridinyl)-1-(4-pyridnyl)-2-propen-1-one known as 3PO became commercially available through Calbiochem, EMD Millipore in 2013. 3PO was shown to suppress glucose uptake and glycolytic flux and was selectively cytostatic to Ras-transformed human bronchial epithelial cells relative to normal cells. Growth of human erythroleukemia cells was inhibited by 3PO [50]. Treatment of tumor-bearing mice reduced the intracellular concentration of fructose 2,6-bisphosphate, glucose uptake and tumor growth. The observation that 3PO suppresses T-cell activation indicates a potential use for small molecule inhibitors of PFKFB3 in the

treatment of autoimmune conditions but might be problematic in combination treatments with immunosuppressive cancer chemotherapeutic agents [56]. A more potent derivative of 3PO designated PFK15 has been identified and a phase I clinical trial is planned [57].

4.3 Pyruvate kinase isoform M2 (PKM2)

Pyruvate kinase (PK) catalyzes the final rate-limiting step in glycolysis (Figure 1), transferring a phosphate group from phosphoenolpyruvate (PEP) to ADP and thereby generating pyruvate and ATP [58]. Humans have four PK enzymes: PKR is restricted to erythrocytes; PKL is found predominantly in liver and kidney; PKM1 is expressed in differentiated somatic cells (e.g. muscle and brain) and PKM2 is found in fetal tissues and proliferating cells. In cancer cells, expression of PKM2 is up-regulated such that it becomes the most predominant PK isoform [59]. Accumulating evidence suggests that PKM2 expression is elevated in cancer cells, as its enzymatic activity can be regulated by various metabolic and signaling inputs [24, 60, 61]. PKM2 influences the fate of glucose carbons (Figure 1). In general, PKM2 activity is low in proliferating cells, which creates a bottleneck at the terminal step in glycolysis, which results in elevated concentrations of upstream glycolytic metabolites. As a consequence, such intermediates are available for the biosynthetic reactions that branch off of glycolysis, thereby increasing the generation of cellular building blocks needed for proliferation [24]

Current models describing the function of PKM2 in cellular proliferation focus mainly on mechanisms that regulate its pyruvate-generating activity as a cytoplasmic component of glycolysis [61, 62]. However, recent studies have provided evidence for activities of PKM2 that extend beyond this canonical role. Namely, several nuclear activities for PKM2, and mechanisms that enable the shuttling of PKM2 into the nucleus, have now been described [54, 63–70]. Nuclear activity promotes tumor growth through the direct transcriptional activation of genes involved in cancer metabolism, including PKM2 itself and the RNA splicing factors that repress PKM1 [54, 65, 68]. In this way, PKM2 acts in a feed forward loop to promote both its nuclear activities and its metabolic role in cancer metabolism. Ongoing studies seek to examine the relative contributions of these two functions in oncogensis and growth.

The central principle presented above asserts that inhibiting PK activity facilitates proliferation. This can be achieved through the expression of the less active and 'regulatable' PK enzyme, PKM2. Alternatively, PKM2 might be upregulated to eliminate PKM1 expression; PKM1 is a constitutively active enzyme, which reduces the generation of carbon for anabolic reactions and facilitates the generation of ATP in the mitochondria. Recent support for this line of thinking comes from the observation that PKM2 is not required for tumor maintenance, where short hairpin (sh)RNA-mediated depletion does not affect tumor growth [71]. Furthermore, genetic experiments, in which the PKM2-specific exon was deleted in a mouse model of breast cancer using Cre-lox technology, demonstrated that the absence of PKM2 increased oncogenesis and negatively affected survival. Strikingly, these tumors showed no tissue-specific PK expression. They did, however, still produce lactate from glucose, suggesting that alternative and non-canonical methods of pyruvate generation were functioning [72]. Collectively, these experiments demonstrate that

PKM2 is not required for oncogenesis or growth, and support for the concept that cancer cells have a growth advantage by removing PKM1.

Despite potential mechanistic differences between these two models on the role of PK in cellular proliferation, they converge on a common point: constitutive PK activity is detrimental for proliferation. Consistent with this concept, the first study to examine the activity of PK directly in an isogenic context demonstrated that cancer cell lines engineered to express PKM1 produce less lactate, consume more oxygen and are less tumorigenic in nude mouse xenografts than those expressing PKM2 [73]. Importantly, these results demonstrated that less PK activity provided a selective growth advantage for cancer cells *in vivo*.

From a therapeutic standpoint, activation of PK may serve as a promising strategy to slow cancer growth. Indeed, numerous research teams in the public and private sectors have recently developed drug-like small molecule activators of PKM2 that make it behave more like PKM1 [74–79]. Studies using such compounds have shown that activation of PKM2 results in decreased accumulation of biosynthetic carbon building blocks and reduced cancer growth [74]. Interestingly, all compounds described thus far share a high degree of structural similarity and bind at the same interface in the PKM2 multimer. The small molecule activator binding pocket is distinct from the fructose bisphosphate (FBP) binding pocket, where PKM2 activators overcome negative regulation by phosphotyrosine peptides. Together, these two activities enable compounds to overcome mechanisms that negatively regulate PKM2.

Several of the compounds described above have been investigated in animal models [74, 75]. In one study, a compound called TEPP-46 activated PKM2 and impaired tumor seeding and growth. Subsequent metabolic analyses support the hypothesis that impaired anabolic metabolism is responsible for the growth inhibition. Future studies are now aimed at exploring the use of these agents in combination with cytotoxic chemotherapies that generate oxidative stress. Support for this concept comes from the observation that activating PKM2 impairs the metabolic control of redox – specifically the generation of reducing equivalents in the form of NADPH and GSH [80] – which sensitizes cancer cells to further oxidative stress. Finally, PKM2 activators may prove to be doubly effective, as multimer formation prevents the nuclear translocation of PKM2 and thus its activity as a protein kinase and activator of gene expression [67].

5. Amino Acid Metabolism

In addition to the well-established role for altered glucose metabolism in cancer, recent research highlights the involvement of amino acid metabolism in cancer, especially glutamine. In proliferating cells, the tricarboxylic acid (TCA) cycle functions as a source of precursors for macromolecular synthesis in addition to generating reducing equivalents for oxidative phosphorylation [81]. Citrate, for example, is both the canonical entry point of the TCA cycle and a precursor for the acetyl-CoA used in fatty acid/sterol synthesis and acetylation reactions. Normally, the withdrawal of citrate to supply these other pathways is

matched by an influx of carbon into the cycle to yield oxaloacetate, refilling the pools of TCA cycle intermediates and maintaining function of the cycle during growth [26].

In culture, many cancer cells meet their anaplerotic demand through the oxidative metabolism of glutamine to oxaloacetate (Figure 2) [26]. Glutamine is an advantageous anaplerotic precursor because its conversion to α-ketoglutarate (α-KG) has the potential to disperse nitrogen to hexosamines, nucleotides, and non-essential amino acids, all of which are required for growth. Furthermore, oxidative metabolism of α-KG to oxaloacetate produces reducing equivalents that can be used to generate energy [82]. Alternative anaplerotic pathways, such as pyruvate carboxylation, also produce oxaloacetate but do not satisfy these other demands, positioning glutamine as a key player in mitochondrial metabolism.

5.1 Glutaminolysis

Glutamine is a non-essential amino acid with an amine functional group and is the most abundant amino acid in circulation [83]. Glutamine supplies nitrogen for nucleobase synthesis and carbon for the TCA cycle, lipid synthesis and nucleotide synthesis [84]. Glutamine is involved in both anabolic and catabolic processes. The catabolism of glutamine is called glutaminolysis, which can be converted into glutamate, aspartate, CO_2, pyruvate, lactate, alanine and citrate. The first step of glutaminolysis is the conversion of glutamine to glutamate and ammonia via glutaminase (GLS). After glutamine is converted into glutamate, the glutamate is oxidized into α-KG. This most often occurs through the enzyme glutamate dehydrogenase (GLDH), concomitant with the generation of mitochondrial NADH or NADPH and ammonia [85]. This is the first energy-yielding step in glutaminolysis and is the link to the TCA cycle (Figure 2).

While glutaminolysis is a normal process for many cells, such as lymphocytes and adipocytes, many cancer cells have elevated glutamine flux. In cell culture, human glioma and HeLa cells were found to be completely dependent upon glutamine for their survival. The cells died in the absence of glutamine, despite being in glucose-rich media, which is now known as the complete dependence on glutamine for cell survival (a.k.a glutamine addiction) [28, 86]. Interestingly, not all cancer cells exhibit glutamine addiction. While the complete molecular mechanism of glutamine addiction is not known, several oncogenic mutations or alterations have been found to explain glutamine-dependence in cancers. For example, Myc is able to increase glutamine metabolism by upregulating GLS expression, which leads to glutamate entry into the TCA cycle as α-KG [87].

The ability to use exogenous glutamine is enhanced by the upregulation of glutamine transporters [88]. For example, one study found the increase in glutaminolysis was so profound that cancer cells accumulated more glutamine than was necessary to meet the energy and anabolic requirements of the cell; excess glutamine-derived carbon was secreted from the cells in the form of lactate or, to a lesser extent, alanine [28]. Further evidence suggests that overexpression of Myc is enough to induce glutamine addiction, due to the fact that this mutation causes the metabolism of the mitochondria to be altered in such a manner as to rely on glutamine despite available glucose [28]. Interestingly, these glutamine-addicted cells were found to be keenly sensitive to electron transport chain inhibitors. This

observation could indicate that either the potential energy in glutamine is being used in the production of ATP [89] or that mitochondrial glutamate uptake by the glutamate/aspartate mitochondrial transporter requires a proton-motive force.

In human pancreatic ductal adenocarcinoma (PDAC), the oncogene *KRAS* contributed to glutamine dependency by using glutamine-derived aspartate to produce oxaloacetate via aspartate transaminase (GOT1) in the cytoplasm. Oxaloacetate was in turn converted into malate and then pyruvate to maintain a high NADPH/NADP$^+$ ratio for redox homeostasis. Therefore, disrupting these reactions, and ultimately glutamine metabolism, led to suppression of PDAC growth *in* vitro and *in vivo* [90].

In a recent study, the use of Nuclear Magnetic Resonance (NMR) Spectroscopy and Mass Spectrometry (MS), as well as stable isotope-resolved metabolomics, allowed the fate of ^{13}C and ^{15}N from labeled glutamine in B lymphoma cells to be traced under aerobic and hypoxic glucose-deprived conditions [91]. In this study, glutamine is the fuel that drives the TCA cycle, which is completely independent of the glucose supply. This reprogramming of the TCA cycle is particularly advantageous to cancer cells under glucose-deprived and/or hypoxic conditions. Where glucose is preferentially converted to lactate under hypoxic conditions, glutamine metabolism serves to sustain ATP production and redox homeostasis in order to support cancer cell growth and survival. This implies that even hypoxic cancer cells can oxidize glutamine through the TCA cycle, and in the absence of glucose, glutamine metabolism alone can sustain the TCA cycle and thereby meet the anapleurotic and energetic demands of the proliferating cancer cells.

Supporting this idea, Myc-transformed cells were found to rely on a means of α-KG synthesis other than that involving GLDH. Aspartate and alanine transaminases reversibly convert glutamate into α-KG, along with oxaloacetate to aspartate or pyruvate to alanine, respectively (Figure 2). These reactions occur despite the presence of GLDH in the mitochondria of cancer cells, which catalyzes the direct conversion of glutamate to α-KG [92]. This is especially important considering it was found that Myc-transformed breast cancer cells undergo apoptosis when aspartate transaminase is inhibited. This implies that this cancer is heavily reliant upon transaminase reactions to produce α-KG [93, 94].

Due to the central nature of glutaminolysis in many cancers, it is becoming an increasingly prominent target in cancer therapy. One of the early strategies to suppress glutamine metabolism was to reduce the amount of available glutamine by using glutamine analogs. Compounds such as 6-diazo-5-oxo-l-norleucine (DON) and acivicin showed cytotoxic effects against several malignant tumors types, including leukemia and colorectal cancers; however these analogs are no longer clinically available due to patient toxicity [95].

More recent strategies have focused on targeting the enzymes of glutamine metabolism. For example, GLS is a potential target for the inhibition of glutaminolysis in cancer cells. The kidney isoform, GLS1, is found in many malignant tumors [96] while the liver isoform, GLS2, is less often expressed in cancers. The compound bis-2-(5-phenylacetimido-1,2,4,thiadiazol-2-yl)ethyl sulfide (BPTES) has been shown to inhibit growth of a variety of cancers in mouse models and in cell culture, including B lymphoma,

which allosterically inhibits GLS1 by altering the conformation of the enzyme [97]. The effect is enhanced under hypoxic conditions, often inducing cancer cell death [91].

GLDH is another potential target of glutaminolysis, which when knocked-down by siRNA resulted in a marked decrease in the proliferation of glioblastoma cells that were glutamine dependent [98]. Green tea polyphenols, hexachlorophene, GW5074, and bithionol may inhibit GLDH function. These inhibitors work by restricting enzyme movement, either by forming rings around the enzyme or wedging between the enzyme's subunits. Currently, green tea polyphenols have been shown to inhibit lung, colon, and prostate adenocarcinoma growth in xenograft models [99]. These compounds also had significant effects on glioblastoma, colon, lung and prostate adenocarcinoma cell proliferation [100].

Another strategy to inhibit glutaminolysis is to target alanine transaminase through L-cycloserine [101] or aspartate transaminase through amino oxyacetate [93] which nearly halted the growth of breast cancer in xenograft mice. Similarly, aspartate transaminase knockdown in pancreatic cancer is also dramatically growth inhibitory in vitro and in vivo and leads to a profound disruption of redox homeostasis [90]. In both of these cases, little to no toxicity was observed with transaminase inhibition in non-neoplastic cells. These studies suggest that aspartate aminotransferase is a promising cancer target. Finally, given that inhibition of aspartate transaminase also leads to a disruption of redox homeostasis, such inhibition may synergize with therapies that increase reactive oxygen species (ROS), such as chemotherapy and radiation [102].

5.2 Reductive Carboxylation

Not all cancer cells have the ability to perform glutaminolysis. Hypoxia limits the oxidative capacity of the TCA cycle, and in particular suppresses production of acetyl-CoA from glucose via pyruvate dehydrogenase (PDH) [103, 104]. Furthermore, some cancer cells contain severe mutations in TCA cycle enzymes (succinate dehydrogenase, fumarate hydratase) or in components of the electron transport chain that prevent efficient production of oxaloacetate from glutamine [105]. Yet, both hypoxic cells and cells with defective mitochondria require glutamine for growth [106–108]. To address this paradox, metabolic labeling experiments were performed using ^{13}C, and revealed that these cells metabolize glutamine through an unusual pathway characterized by "reversal" of isocitrate dehydrogenase (IDH) enzyme activity (Figure 2, blue arrows) [106, 107, 109, 110]. IDH typically acts as an oxidative decarboxylase, converting isocitrate to α-KG and CO_2 in the presence of an electron acceptor. Indeed, IDH3, the mitochondrial NAD$^+$-dependent IDH isoform, functions exclusively in this manner. However, the two other mammalian IDH isoforms, IDH1 and IDH2, use NADP$^+$/NADPH as cofactors, and can act either as oxidative decarboxylases or reductive carboxylases. In the latter reaction, α-KG is carboxylated to produce isocitrate, converting NADPH to NADP$^+$. Isocitrate is readily converted to citrate, which can then be cleaved to produce acetyl-CoA. Under conditions of reductive carboxylation, glutamine becomes the major source of acetyl-CoA for fatty acid synthesis, greatly decreasing the need to produce acetyl-CoA from glucose. Furthermore, citrate cleavage yields oxaloacetate, which is converted to other 4-carbon intermediates, meaning that essentially the entire cellular pool of TCA cycle intermediates can be derived from

reductive carboxylation, in reverse order to their conventional route of production (Figure 2).

Reductive carboxylation had previously been observed as a minor source of isocitrate and citrate in a number of non-transformed mammalian tissues [111–114]. Its importance in cancer cell biology is related to its ability to serve as the major source of citrate and acetyl-CoA when cellular circumstances conspire to inactivate pathways that normally produce these metabolites, including hypoxia and genetic reprogramming of mitochondrial metabolism. For example, the Von Hippel-Lindau (VHL) tumor suppressor normally functions in the oxygen-dependent degradation of the alpha subunits of the hypoxia inducible factor (HIF) transcriptional activators. Thus, in cells expressing VHL, oxygen facilitates the degradation of HIF1α and HIF2α so that HIF target genes are not expressed. In contrast, in malignant tumor cells lacking VHL, HIF target genes are expressed regardless of oxygen availability. These genes, which include glucose transporters and glycolytic enzymes, are part of the metabolic adaptation to hypoxia. Importantly, hypoxia stimulates the expression of PDH kinase-1 (PDK1), which phosphorylates and inactivates the PDH complex, impairing its ability to provide acetyl-CoA and citrate from glucose [103, 104]. Cancer cells lacking VHL produce a substantial fraction of citrate and fatty acids using glutamine-dependent reductive carboxylation in culture, and a small but detectable fraction of citrate in vivo [115]. Heterologous expression of wild-type Vhl, or silencing of PDK-1, partially reverts metabolism to a phenotype in which citrate and fatty acids are produced from glucose/PDH [115].

Regulation of reductive carboxylation is an area of active investigation. Importantly, the pathway is stimulated by mutations in the electron transport chain that impair recycling of mitochondrial NADH to NAD^+, but importantly NADPH is the cofactor for reductive carboxylation. This suggested a model in which a low NAD^+/NADH ratio in mitochondria provides a source of reducing equivalents which are transmitted to NADPH by nicotinamide nucleotide transhydrogenase (NNT) [107]. NNT is an inner mitochondrial membrane protein that uses the electrochemical proton gradient to transfer reducing equivalents from NADH to NADPH [116]. Silencing NNT expression in SkMel5 melanoma cells reduced the contribution of glutamine carbon to TCA cycle intermediates through both oxidative and reductive metabolism, and decreased the rate of growth of SkMel5-derived subcutaneous xenografts [117]. In VHL-deficient 786-O renal carcinoma cells, which produce a large fraction of citrate via reductive carboxylation, NNT silencing significantly suppressed reductive glutamine metabolism. These findings suggested that NADPH produced by NNT is required for reductive carboxylation in VHL-deficient cells.

Reductive carboxylation is also regulated by changes in the abundance of TCA cycle metabolites. Citrate levels tend to be low in cells with active reductive carboxylation, and maneuvers to suppress formation of citrate from glucose enhance the fraction of citrate derived from reductive carboxylation [106, 107, 109, 115]. By contrast, supplying cells with exogenous acetate or citrate increases the ratio of citrate/α-KG while reducing the overall contribution of reductive carboxylation to TCA cycle metabolism [115].

Compartmentalization of the reductive carboxylation reaction is not well understood. IDH1 is cytosolic while IDH2 is localized to the mitochondria, and data suggest that each isoform can participate in reductive carboxylation. IDH2 was found to be the crucial isoform for reductive carboxylation in hypoxic glioma cells [109], whereas other cell lines required IDH1 under hypoxia [106]. Genetic suppression of pyruvate dehydrogenase in lung cancer cells is sufficient to induce a net flux of reductive carboxylation, and under these circumstances the flux is completely dependent on IDH1 [118]. In cells with mutations in the electron transport chain, silencing either isoform reduced activity of the pathway, and silencing both together had a maximal effect [107]. The involvement of NNT as a source of NADPH argues for mitochondrial localization of the reductive carboxylation reaction, at least in those cells that require NNT expression to maintain citrate levels. However, cytosolic sources of NADPH, including the oxidative branch of the pentose phosphate pathway, may play a more prominent role in cells where IDH1 catalyzes reductive carboxylation. Presumably, the choice of isoform is also related to localization of an available source of the substrate α-KG, meaning that compartmentalization of glutamine metabolism could add another dimension to regulation of this pathway.

6. Lipid Metabolism

Cancers may use a wide variety of substrates and substrate sources to meet their catabolic and anabolic needs, including internally- and externally-derived fatty acids (FAs). Indeed, FAs are essential for cellular proliferation. In particular, FAs are used as cellular building blocks for lipid membrane synthesis, for energy storage and production, as well as for cellular signaling. Thus, limiting abundance of FAs could be a therapeutic strategy against cancer. Limiting cellular FAs could be achieved by: blocking synthesis (lipogenesis), increasing breakdown (oxidation), reducing availability from storage (lipolysis), or by increasing FA flux towards storage (re-esterification); these distinct strategies have been considered as chemotherapeutic strategies recently [119]. As with other metabolic shifts in cancer, characterizing the state of lipid metabolism in unique cancer types and cells lines is an important first step. Indeed, successful chemotherapeutic strategies will require understanding the specific abnormalities in lipid metabolism for a given cancer type. Targeting lipid metabolism in cancer is an emerging idea that warrants further investigation.

7. Epigenetics and Oncometabolites

In addition to alterations in metabolism, metabolic reprogramming mediated by specific oncogenes and tumor suppressors can impact dynamic regulation of chromatin via post-translational modifications. Indeed, increasing evidence indicates that altered metabolism can also lead to changes in nutrient-sensitive post-translational modifications. These chemical modifications, such as O-GlcNAcylation, methylation, and acetylation, can impact the activities of metabolic enzymes, signaling components, transcriptional regulators, and chromatin-associated proteins such as histones [120–124]. Furthermore, oncometabolites, such as 2-hydroxyglutarate (2-HG), can have profound consequences on the regulation of chromatin organization, gene expression, and genome integrity. Improved understanding of the links between metabolism and epigenetics is expected to have important therapeutic

implications, and intense efforts are currently geared towards targeting both altered metabolism and epigenetics [125, 126].

7.1 O-GlcNAcylation

The hexosamine biosynthetic pathway (HBP), a branch of metabolism that diverges from glycolysis at fructose-6-phosphate, generates uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), a key donor substrate for glycosylation reactions, including the *O*-GlcNAc modification, which is mediated by *O*-GlcNAc transferase (OGT) [122, 127, 128]. *O*-GlcNAcylation is generally elevated in cancer cells and has been linked to malignant tumor growth through direct modification of phosphofructokinase (PFK1) and indirect regulation of FoxM1 [129–131]. All four histones (H2A, H2B, H3, and H4) are modified by *O*-GlcNAc, which is dynamically regulated in response to nutrient availability [132–134]. *O*-GlcNAcylation of histone H3 on Ser10 is cell cycle regulated and suppresses mitosis-specific H3 phosphorylation [135], whereas H2B Ser112 *O*-GlcNAcylation facilitates monoubiquitylation of Lys120 of histone H2B, a mark associated with active transcription, indicating a role for *O*-GlcNAc in gene regulation through chromatin modification [134]. OGT can also associate with Ten-Eleven-translocation (TET) family 5-methylcytosine hydroxylases and participate in TET-mediated gene regulation [136–139]. Whether cancer cells exhibit altered histone *O*-GlcNAcylation patterns due to metabolic alterations is not yet clear. Notably, TET2 function is frequently disrupted in hematopoietic malignancies [140–142], and future studies will determine whether changes in histone *O*-GlcNAcylation participate in cancer development in these cases.

7.2 Methylation

Alterations in histone and DNA methylation are widely observed in cancer. Many cancer types display global DNA hypomethylation compared with normal tissue, while genes regulating cell-cycle and DNA damage response are frequently found to be hypermethylated, and thus silenced [143]. Histone and DNA methyltransferases utilize the methyl donor *S*-adenosyl methionine (SAM), which is synthesized from methionine and ATP. Hence, SAM availability for methylation reactions may be sensitive to levels of methionine taken up from the environment or synthesized through one-carbon metabolism pathways, discussed in more detail below. In yeast, SAM can serve as a sensor of amino acid availability, inhibiting autophagy and promoting growth [144]. While transformed cells require growth medium supplemented with methionine [145], knowledge of how cancer cells use methionine to regulate histone and DNA methylation is still limited. Notably, the enzyme nicotinamide N-methyltransferase (NNMT), which depletes SAM by catalyzing the transfer of SAM's methyl group to nicotinamide, is frequently overexpressed in human cancers. A recent study demonstrated that NNMT levels impact methylation in cancer cells, with NNMT overexpression associated with reduced levels of SAM and histone methylation [146]. Interestingly, metabolic regulation of SAM production has also been implicated in modulating histone methylation levels and maintaining pluripotency in mouse embryonic stem cells [147]. In addition to overall cellular levels of SAM, localized pools of SAM may provide an additional layer of metabolic control. The enzyme methionine adenosyltransferase (MATIIα, which produces SAM, has been localized to gene promoters and implicated in gene regulation, indicating that enzymatic production of metabolites can

be targeted to specific loci and coupled to transcriptional regulation [148]. DNA and histone methylation levels are also controlled by rates of demethylation. Metabolic control of demethylation is discussed below in the "Oncometabolites" sub-section. Given the substantial evidence that DNA and histone methylation abnormalities contribute to cancer initiation and growth, further investigation into the role of metabolism in determining methylation changes in cancer is needed.

7.3 Acetylation

Lysine acetylation levels are determined by the combined actions of lysine acetyltransferases (KATs) and deacetylases (HDACs), both of which may be influenced by metabolic state. Histone proteins are acetylated at multiple lysines, and these modifications are involved in chromatin-dependent processes, including gene transcription, DNA replication, and DNA damage repair. In mammalian cells, nuclear-cytosolic acetyl-CoA, the donor substrate for acetylation reactions, is generated either through the cleavage of citrate by ATP-citrate lyase (ACLY) or directly from acetate by acetyl-CoA synthetase (AceCS1) [122]. Histone acetylation can be regulated by the availability of glucose or acetyl CoA [149–152], and in mammalian cells glucose-dependent regulation of histone acetylation occurs in an ACLY-dependent manner [149]. Reciprocally, nuclear-cytoplasmic lysine deacetylation can be mediated by SIRT1, a member of the NAD^+-dependent sirtuin family of lysine deacetylases [class III histone deacetylases (HDACs)] [153, 154]. NAD^+ levels rise under nutrient-restricted conditions, in part mediated by AMP-activated protein kinase (AMPK) [155, 156]. In addition, the ketone body β-hydroxybutyrate (βOHB) was recently demonstrated to function as an endogenous inhibitor of class I HDACs under ketogenic conditions and influence the state of histone acetylation and gene transcription [157].

Metabolic control of histone acetylation is likely to impact cancer growth, although this has not yet been directly shown. In yeast, acetyl-CoA acts as a growth signal, promoting histone acetylation at and expression of growth-related genes [158]. Levels of ACLY are frequently elevated in cancer [159], although the impact of this on overall histone acetylation is not yet known. Global histone acetylation levels are highly heterogeneous among cancers, and several studies have shown correlations with cancer recurrence and patient survival [160–164]. Both KAT and HDAC inhibitors have shown promise in cancer therapy [125], although mechanisms of action remain poorly understood.

7.4 Sirtuin deacylases (SIRTs)

The sirtuins (SIRT1-7) are a class of conserved NAD^+-dependent deacylases that control cellular metabolic processes and protect the cell against metabolic and genotoxic stresses. Not surprisingly, altered regulation of the sirtuins is associated with many diseases such as diabetes, neurodegenerative diseases, obesity, and cancer [165]. Rapidly proliferating cancer cells require shifts in metabolism that promote anabolic metabolism, as such the sirtuins play an important role in controlling cancer development and progression by maintaining normal cellular metabolism. Of the 7 mammalian sirtuins four of them, SIRT1, SIRT3, SIRT4, and SIRT6 have been associated with various types of cancer and are generally believed to be associated with cancer cell metabolism and control of the Warburg effect as well as DNA damage responses. SIRT1, SIRT3, and SIRT6 are strong deacetylases, while

the enzymatic activity of SIRT4 is less clear but it has been shown to be a weak ADP-ribosyltransferase and possibly a weak deacetylase [166]. SIRT1 and SIRT6 serve to mainly deacetylate histones and transcription factors in the nucleus, while SIRT3 and SIRT4 are mitochondrial, where they act on metabolic proteins [167].

SIRT1 shares the most homology to Sir2 (*S. cerevisiae*), the first sirtuin identified, and has been shown to increase lifespan and protect against age-related diseases. The main targets of SIRT1 are the transcription factor peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1α), the FOXO family of transcription factors, and the tumor suppressor p53 [168]. Studies have found that SIRT1 expression is elevated in certain cancers and repressed in others, making the role of SIRT1 in cancer difficult to define [169]. It seems as though the main confusion underlying the role of SIRT1 in cancer is the complexity behind the SIRT1-mediated inhibitory deacetylation of p53. SIRT1 expression is also positively regulated by p53 in an apparent negative feedback loop where loss of p53 reduces SIRT1 expression, leading to an increase in p53 activity [170]. SIRT1 has been described as a tumor suppressor that promotes the DNA damage response, and $SIRT1^{+/-}$ $p53^{+/-}$ mice develop cancer in a variety of tissues, particularly sarcomas. Furthermore, SIRT1 overexpressing mice are more metabolically efficient, resistant to diabetes, and no cancer phenotype has been reported in these mice [171]. SIRT1 expression is reduced in various human cancers, particularly breast cancer and hepatocellular carcinoma [172]. Activation of SIRT1 has already proven beneficial in BRCA1-associated breast cancers as treatment with resveratrol activated SIRT1 and inhibited cell proliferation in vitro and tumor growth *in vivo* [173]. It seems as though activation of SIRT1 may have therapeutic potential in certain cancers in order to normalize cellular metabolism and improve the DNA damage response.

SIRT3 is the main mitochondrial deacetylase and as such it controls the activities of many metabolic enzymes in the mitochondria. SIRT3 deacetylates a long list of mitochondrial proteins acting at several nodes of mitochondrial metabolism, including fatty acid oxidation, glutamine metabolism, and mitochondrial ROS production. When SIRT3 is lost or ablated, metabolic derangements occur [174], which may be the source for the link between SIRT3 and cancer. The first described link between SIRT3 and cancer reported that SIRT3KO mice spontaneously develop mammary tumors after two years [175]. Furthermore, this study identified SIRT3 as a tumor suppressor by showing that SIRT3 KO MEFs could be transformed *in vitro* by the addition of a single oncogene, Myc or Ras [175]. A later study attributed the elevated level of cellular ROS seen with loss of SIRT3 to the stabilization of HIF-1α and activation of HIF-1α glycolytic target genes, leading to altered cellular metabolism towards glycolysis [176]. It is possible that SIRT3 deficiency leads to a cancer permissive state by coordinating a shift in metabolism to a Warburg phenotype.

Cancer cells also rely heavily on the metabolism of glutamine as a nitrogen source for protein and nucleotide synthesis necessary for proliferation [28]. SIRT4 was previously found to inhibit the activity of glutamate dehydrogenase (GDH) activity by ADP-ribosylation. More recently, one study showed that genotoxic stress causes an induction of SIRT4 expression leading to a repression of glutamine metabolism [177]. SIRT4 KO MEFs had higher glutamine uptake in response to UV damage and increased glutamine-dependent

proliferation compared to wild type cells. Finally, SIRT4KO mice had increased incidence of various types of cancer, particularly lung cancer compared to wild type mice [177].

Finally, SIRT6 controls cancer metabolism and SIRT6 ablation activates aerobic glycolysis and leads to oncogenesis, in the absence of oncogene activation [178]. This study showed that when aerobic glycolysis was inhibited by inhibition of PDK1, an inhibitor of pyruvate dehydrogenase (PDH), the SIRT6 deficient cells failed to form tumors, showing a reliance on glycolytic metabolism of glucose. Further, SIRT6 expression is also low in pancreatic, colon, and liver cancers in humans [178, 179]. Overall, it seems as though SIRT6 suppresses cancer growth by regulating aerobic glycolysis and that a mutator phenotype is not required for SIRT6-dependent tumor formation.

Collectively, these studies show that the loss of an individual sirtuin can promote oncogenesis by creating a state that is favorable to cancer cells, such as increasing DNA damage, inhibiting DNA repair mechanisms, or altering cellular metabolism towards a Warburg-like phenotype. Modulating sirtuin expression may be a way to alter substrate use by cancer cells and slow or prevent their growth. Many of the sirtuins are known to be regulated by nutrient status, therefore it may be beneficial to modulate sirtuin expression by diet and/or exercise. SIRT1 and SIRT3 expression are induced with calorie restriction and fasting, and SIRT3 expression is reduced with high fat diet (HFD) feeding [180, 181]. Modulating sirtuin expression through diet, and possibly exercise, may be useful in combination with conventional cancer therapy. By combining chemotherapy with calorie restriction or exercise (described below), sirtuin expression may be elevated leading to a more normalized cellular metabolism and better control of the cancer, possibly increasing the efficacy of the drug therapy. Along these lines, efforts aimed at identifying calorie restriction mimetics, similar in action to resveratrol, have the overall goal of increasing the expression or activity of the sirtuins. However, resveratrol, other CR mimetics, and dietary manipulations are not selective for a particular sirtuin and may have undesired or off-target affects, and most importantly may not activate the sirtuins robustly. More investigation into the sirtuins in cancer is clearly needed, and better ways to selectively target individual sirtuins will be key moving forward.

7.5 One-carbon metabolism

The folate cycle in combination with the methionine cycle are collectively referred to as the one-carbon metabolism, since carbon units are circulated through multiple enzymatic reactions. The one-carbon cycle forms a critical component of the cellular metabolic network, which is linked to nearly all of the major biosynthetic pathways. The main sources that put carbon units into this cycle are serine and glycine biosynthetic pathways. Importantly, the fate of the carbons (i.e. outputs) of the one-carbon cycle consist of a variety of critical metabolic pathways; for example, some outputs include nucleotide metabolism [182], protein translation [183], lipid metabolism [184], methylation metabolism [121, 123, 185–187], protein sulfhydration, glutathione production. Therefore, the activity of the one-carbon cycle is important in regulating the biosynthesis of the building blocks of a cell as well as its epigenetic status. Furthermore, the redox state of the cell is regulated by this cycle at two levels: through the folate cycle by the function of tetrahydrofolate (THF), and also

through the glutathione production via the transulfuration pathway that mediates the levels of ROS in cells. Both of these mechanisms serve to regulate the balance of NADPH/NADP$^+$ levels in the cell [24]. Recently, a variety of cancers exhibiting the Warburg effect were shown to divert carbons from glycolytic metabolism into the one-carbon cycle, providing a link from the Warburg effect to the activity of one-carbon metabolism [188, 189].

Studies have shown that phosphoglycerate dehydrogenase (PHGDH) is hyperactivated in a subset of cancers, resulting in over-production of serine in these cancers [190, 191]. Furthermore, the gene encoding PHGDH was shown to have undergone significant copy number gain in some cancers and cancer cell lines, and these cell lines were dependent on the *PHGDH* amplification for rapid proliferation [192].

A second instance where one-carbon metabolism has shown correlations with cell transformation is the metabolism and generation of glycine. A metabolomics study on cancer cell lines reported a strong association between glycine uptake and catabolism with cancer cell proliferation [193]. Also another study found that ectopic expression of glycine dehydrogenase (GLDC, decarboxylating), phosphoserine aminotransferase (PSAT), and serine hydroxymethyltransferase (SHMT), all enzymes important in glycine uptake and catabolism, could induce cell growth in NIH 3T3 cells, thus conferring to these cells properties indicative of the hallmarks of cancer [194].

Additional levels at which the one-carbon cycle and its output pathways have been linked to cancer include nucleotide metabolism, epigenetic modifications, polyamine metabolism, and the regulation of the oxidative state of the cell. Multiple genes involved in nucleotide metabolism have been shown to be able to cause transformation by reducing the genomic integrity [195]. Also, through the transfer of methyl groups to proteins, DNA, and RNA, *S*-Adenosyl Methionine regulates protein activity as well as epigenetic marks on DNA and proteins in cells which are all broadly implicated in cancer.

Targeting one-carbon metabolism using folate antagonists has long been used as a major class of cancer therapeutic agents [196]. Historically, aminopterin was the first one of such drugs, and methotrexate and pemetrexed are still being used as common chemotherapeutic agents in a variety of cancers acting to inhibit di- and tetrahydrofolate reductase activities, thereby disrupting the one-carbon cycle. In addition to this class of compounds, another major group of chemotherapeutic agents linked to the one carbon cycle are inhibitors of nucleotide metabolism. These include 5-fluorouracil (5-FU) and gemcitabine [197].

Furthermore, several novel anti-cancer drugs that are currently being tested in clinical trials also target specific components of the one carbon cycle. These agents mostly target the epigenetic state of the cancer cells through inhibiting SAM or DNA methyltransferases or polyamine synthesis [198]. Difluromethylornithine (DFMO), methylglyoxal bis(guanylhydrazone) (MGBG), and an inhibitor of *S*-adenosylmethionine decarboxylase called SAM486A are examples of such agents.

One carbon metabolism consists of enzymes that are in principle druggable [199]. Therefore, several promising drug targets include PHGDH, PSAT, PSPH, GCAT, GLDC, and glycine N-methyltransferase (GNMT). A recent study has shown that reduction in serine

and glycine intake in diet can significantly reduce cancer cell proliferation in mice, suggesting that one carbon metabolism can potentially be targeted through dietary adjustments [200]. Finally, due to the prevalence of anti-metabolite chemotherapeutic agents that are somehow involved with the one carbon cycle, expression levels of some of the components of this pathway could potentially be used as biomarkers for drug selection and outcome prediction in several types of cancers [201, 202].

7.6 Oncometabolites

IDH1 and *IDH2* are frequently mutated in several cancer types, including glioma, acute myeloid leukemia, and chondrosarcoma [203–205], resulting in significant changes to the epigenetic landscape in cancers harboring these mutations [120, 141, 206, 207]. The products of wild type IDH1 and IDH2 enzymes, NADPH and α-KG, play broad functions in regulating cellular metabolism. The mutant IDH proteins lose their wild type function and instead convert α-KG into (*R*)-2-hydroxyglutarate [(*R*)-2HG], a structural analog of α-KG [208]. IDH mutant cancer cells can accumulate millimolar levels of this normally undetectable metabolite [208, 209]. Recently, mouse models with altered IDH2 activity have shown the capacity of IDH mutations to facilitate malignant tumor development and maintenance [210, 211].

(*R*)-2HG is thought to act by competitively inhibiting certain enzymes that require α-KG as a cofactor, including Jumonji-C domain histone demethylases (JHDMs) and TET proteins, which are implicated in DNA demethylation [212, 213]. IDH mutant tumors display a hypermethylation signature, associated with a block in cellular differentiation [120, 141, 212]. A similar hypermethylation signature is associated with IDH mutations and TET2 mutations, which occur in a mutually exclusive manner in AML, indicating that these mutations likely target the same pathway [141]. Moreover, either treatment with (*R*)-2HG or silencing of TET2 was sufficient to promote growth factor-independent growth of TF-1 leukemia cells [214]. However, the epigenetic alterations mediated by (*R*)-2HG may not fully explain its cancer promoting effects, since (*S*)-2HG, which is not produced by mutant IDH but serves as an even more potent inhibitor of TET2 than (*R*)-2HG, fails to transform TF-1 cells. One possible explanation is that (*R*)-2HG, but not (S)-2HG, can act as an agonist for EGLN prolyl hydroxylases, which promote hypoxia-inducible factor (HIF) degradation; hence regulation of HIF proteins may also be a part of the malignant tumor-promoting activity of (*R*)-2HG [214, 215]. Indeed, EGLN1 silencing impaired the ability of IDH1 R132H to transform TF-1 cells.

2HG has emerged as a useful diagnostic marker for patients with IDH mutant tumors. Patients with IDH mutant AML exhibit elevated serum 2HG levels, and those with the highest levels of 2HG had shorter overall survival [216]. Moreover, 2HG can be detected noninvasively by magnetic resonance spectroscopy in patients with IDH mutant glioma [217]. Substantial interest has also arisen in therapeutic targeting of mutant IDH enzymes, as a cancer-specific metabolic alteration. Inhibitors to mutant IDH1 and IDH2 were recently reported. For example, an IDH2/R140Q inhibitor promoted the differentiation of leukemia cells containing that mutation [218]. An IDH1/R132H inhibitor likewise promoted differentiation and reduced histone methylation in glioma cells. Growth of glioma

xenografts was also impaired. Notably, the tumor growth inhibitory effects of the drug were observed at lower doses than were required to stimulate histone demethylation and differentiation, further suggesting that additional mechanisms may contribute to mutant IDH-mediated malignant tumor growth [219].

In addition to (*R*)-2HG, succinate and fumarate also have the potential to act as oncometabolites. In specific cancer types, loss-of-function fumarate hydratase (FH) and succinate dehydrogenase (SDH) mutations are observed, resulting in build-up of fumarate and succinate, respectively. Similar to 2HG, fumarate and succinate can competitively inhibit α-KG-dependent dioxygenases. As such, succinate has been known for several years to promote HIF1α stabilization via inhibition of prolyl hydroxylases [220]. More recently, it has been identified that succinate and fumarate also inhibit histone demethylases and TET proteins [221]. Both SDH mutant gastrointestinal stromal tumors and paraganglioma were shown to exhibit a hypermethylation phenotype [222, 223].

7.7 Lactate

As described above, lactate is made from pyruvate by the enzyme lactate dehydrogenase (LDH) during normal cellular metabolism (Figure 1). Cancer cells produce high levels of lactate, as described above. More recently, a new role for lactate has been described wherein some cancer cells use lactate to enable proliferation. For example, in a cancer microenvironment, excess lactate is secreted and contributes to an extracellular environment that promotes cancer progression [224]. Thus, lactate, which was previously considered a waste product of cancer cells, has now been identified as a key metabolite that plays a direct role in promoting cellular growth. The concept of the role of lactate as a signal or as a fuel in cancer is often called the "reverse Warburg effect" [225].

Lactate levels are governed by a number of factors, including differential expression of LDH isoforms, the lactate monocarboxylate transporter (MCT) levels, and oxidative capacity of tissues. LDH is the primary enzyme catalyzing lactate turnover, which interconverts pyruvate and lactate, with concomitant interconversion of NADH and NAD$^+$, respectively. LDH is involved in the metabolism of both glucose and glutamine carbon, as well as in determining malignant tumor pH and the activity of the TCA cycle [226]. LDH is further classified into five different subtypes (LDH1-5), which structurally conform into homo- or hetero-tetramers of M protein subunits encoded by LDH isoform A (LDHA) and H protein subunits encoded by LDH isoform B (LDHB) genes. These subtypes vary based of tissue distribution. For example, in normal tissue, LDH1 has high expression in the brain, heart, and kidney; LDH5 is found in glycolytic tissues, such as liver or skeletal muscle [227].

Recently, LDHA was shown to be required for the maintenance and progression of many cancers [228–231], but the mechanisms by which LDHA facilitates cancer progression remain poorly understood. Indeed, targeting lactate metabolism is re-emerging as a therapeutic strategy [232]. Given the correlation between LDHA levels and poor outcomes, LDHA has attracted attention as a cancer target. Reducing LDHA levels (by shRNA) in cells leads to decreased proliferation and suppressed oncogenicity [228]. Furthermore, inhibition of LDHA pharmacologically (FX11) or by siRNA reduces ATP levels and results in cellular death [229]. A follow-up study examining the combination treatment of FX11 (LDHA

inhibitor) and FK866 (an NAD$^+$ synthesis inhibitor) resulted in lymphoma regression in a xenograft model [229]. Another study identified that LDHA plays a role in chemoresistance in breast cancer [233]. Specifically, paclitaxel-resistant breast cancer cells have high expression and activity of LDHA compared to paclitaxel-sensitive cells. Importantly, downregulation of LDHA restores chemosensitivity, indicating that lactate dehydrogenase, and more broadly lactate levels, play a key role in cancer drug resistance and may serve as a therapeutic target.

In addition to LDH, MCTs have come under increased scrutiny for their ability to import and export lactate. This family of transporters moves lactate and a proton down a concentration gradient, which determines the directionality of transport. Similar to LDH, different MCT isoforms are expressed in tissue types according to their oxidative capacity [234, 235]. For example, MCT1 is high in oxidative cell types like muscle sarcolemma [236], and increases with training. MCT4, on the other hand, is found in cells with high rates of glycolysis, such as fast-twitch muscle cells. Consistent with this idea, its expression is increased during hypoxia.

MCTs have also garnered interest as regulators of lactate metabolism. Indeed, inhibition of MCT1, either pharmacologically with α-cyano-4-hydroxycinnamate (CHC), or by RNA interference, shifted metabolism from lactate consumption to glucose consumption in normoxic cells. Interestingly, this also revealed a metabolic flexibility of cancer cells. Some studies have also tested MCT1 inhibition *in vivo.* In one mouse model of lung cancer and in a xenograft of human colorectal adenocarcinoma, CHC administration reduced hypoxia, induced tumor necrosis, and slowed overall growth. In another study, CHC was able to induce anti-tumoral and anti-angiogenic activity in gliomas, as well as to potentiate the effect of the alkylating agent temozolomide [237].

To conclude this section on emerging metabolic derangements contributing to cancer cell growth, we end with lactate – Warburg's original observation. Collectively, these studies support a model where metabolites, like lactate, which facilitate malignant tumor development and/or survival, have the potential to be targeted therapeutically.

8. Therapeutic Strategies

Metabolism is a recent addition to the hallmarks of cancer, and the nature of these changes and their significance for the etiology of the disease are areas of intense investigation. As in other reviews of 'hallmarks of cancer' in this special issue, both molecular targets and small molecules were evaluated as lead candidates to influence different aspects of cancer metabolism. Each of the priority targets was outlined in the preceding sections and together are summarized in Table 1. These areas were chosen for their known or emerging mechanisms to contribute to cancer metabolism.

Next, a team of researchers consisting of specialists in each hallmark area performed an extensive literature search to determine if any studies had been performed addressing the effect of each molecular target on other hallmarks. The 'Target Validation Team' also looked for any possible reports of effects by the identified therapeutic approaches on other hallmarks. Given the interest in future research that will focus on combination approaches,

this effort was primarily aimed at highlighting any evidence of contrary effects in other hallmark areas. However, any potential for complementarity or synergy were also considered as well.

Remarkably, many targets described above as having potential to manipulate different aspects of cancer metabolism have not been directly tested in the context of other hallmarks. In particular, compounds need to be tested for their influence on other hallmarks, independent of their effects on metabolism; this is the current major gap in knowledge. We primarily attribute this to the recent resurgence in metabolic studies, especially in the context of cancer. Indeed, less is known about if and how to effectively target cancer metabolism as a therapy than is known about targeting other hallmarks of cancer. Thus, our analysis at this time shows that prioritized targets and lead compounds that might affect these targets need further investigation. With more research, a broad-spectrum approach to target metabolism and to synergize with other hallmarks of cancer could be achieved.

In the interim, based on current knowledge, we consider from a drug development perspective the unique environment of mitochondria, which needs to be considered to target mitochondrial function, and ultimately manipulate metabolism. Because of the challenges associated with targeting mitochondria, we also take a step back from the cellular and biochemical mechanisms of metabolism and discuss how alterations in host metabolism could more broadly manipulate cancer metabolism in an efficacious way. Together, considering both cancer (direct) and host (indirect) metabolism could uncover novel therapeutic strategies or information that could be leveraged to target metabolism.

8.1 Strategies To Target Mitochondria In Cancer

Traditionally, mitochondria were not thought of as a 'druggable target' due to significant challenges unique to mitochondria, including selective delivery to this organelle, specific mitochondrial uptake within a targeted organ, and how to enhance the selective delivery to mitochondria within cancer cells; however, recent work is changing this view [238, 239]. In part, this is due to the growing realization that mitochondrial function contributes to multiple cellular functions, beyond energy metabolism, and these aspects could be targeted. The development of strategies to target different aspects of mitochondrial biology has led to the emergence of a new field called mitochondrial pharmacology [238, 239].

In mitochondrial pharmacology, development of drugs designed to affect mitochondria require the following considerations. Foremost, mitochondria originate from the coordination of two genomes, with 37 genes in the mitochondrial DNA and approximately 1,500 proteins being imported into the mitochondria following their transcription and translation from nuclear genes [240]. The expression of the two genomes is coordinated by a number of nuclear transcription factors [241]. Targeting nuclear- versus mitochondrial-derived proteins could be important. Furthermore, the mitochondria themselves continually join and separate through fission/fusion processes that are linked to the turnover and degradation of damaged, defective or surplus mitochondria [242]. Thus, one way for drugs to alter mitochondrial function is through interaction with the cellular machinery that controls overall mitochondrial biogenesis. For example, drugs such as 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) that interact with the AMP-activated protein kinase

(AMPK) [243] may lead to upregulation of global mitochondrial biogenesis. Furthermore, agonists for PGC1α, a transcriptional co-activator that regulates gene expression of nuclear encoded mitochondrial proteins may do the same [244]. Such strategies may globally alter mitochondrial nutrient oxidation or function in therapeutically useful ways in cancer.

More direct strategies have been demonstrated by using drugs that act on particular targets within mitochondria. For example, dichloroacetate has been explored as an anticancer agent by blocking the activity of the inhibitory kinase that inactivates pyruvate dehydrogenase, thereby enhancing mitochondrial pyruvate oxidation [245]. Generally, in these examples, a drug acts on an aspect of metabolism that is mitochondrial and the selectivity comes from the interaction of the drug with its target, which happens to be in the mitochondrion. However, emerging methods are available to target compounds to mitochondria [238], and these may have a number of advantages. In particular, the concentration of a compound within mitochondria greatly enhances the potency of the therapy, meaning far less of a compound might be required. The localization of the compound within the mitochondria also minimizes metabolism and toxicity associated with reactions elsewhere in the body.

An important attribute in designing targeted therapies for mitochondria is the distinct mitochondrial compartments, with dramatically different properties. Most of energy metabolism occurs in the matrix and on the mitochondrial inner membrane. The intermembrane space has limited metabolic activity, although it is important in protein import; this compartment contains many of the proteins that are released into the cytoplasm during activation of apoptotic pathways. The mitochondrial outer membrane is also a key component in the regulation of apoptosis. Furthermore, many of the complexes involved in the propagation and stabilization of inflammatory pathways are assembled on the cytoplasmic-facing surface of the mitochondrial outer membrane [246].

Overall, the role of mitochondria in cancer is becoming better understood, suggesting that therapies targeted to the organelle may be useful [247, 248]. Drugs could be designed to affect mitochondrial function directly by binding to targets within mitochondria, or indirectly by altering mitochondrial biogenesis. Recent developments have also led to robust strategies to target bioactive compounds to mitochondria. These approaches may selectively modify mitochondrial metabolism in cancer cells to generate therapeutically beneficial outcomes, such as enhancing the selective killing or inhibiting growth of cancer cells. While many limitations and uncertainties with these approaches remain, modifying mitochondrial function could become a therapeutically useful extension of current cancer treatments.

8.2 Strategies To Target Metabolic Interactions Between Cancer And Host

Cancer metabolism might be manipulated specifically through host metabolism. This rationale is based on the concept that pharmacological mimetics of weight loss, calorie restriction, and/or targeting the associated hormonal systems and intracellular signaling pathways are emerging pathways for novel cancer therapeutics. Indeed, one of the possible mechanisms underlying known efficacy of fasting, CR, exercise, or weight loss on cancer could be multi-faceted, synergistic effects of these "treatments". Thus, manipulating metabolism in these ways could be a successful therapeutic strategy. Additionally, further studying *physiological* approaches that influence a broad-spectrum of targets and/or

hallmarks could inform more effective design of *pharmacological* approaches aimed at a broad-spectrum of targets.

8.3 Calorie Restriction, Fasting, and Cancer

Calorie restriction (CR), usually referring to a 20%–40% reduction in the daily intake of calories without malnutrition has been shown to extend lifespan and healthspan in multiple species, in part by delaying or preventing the occurrence of many age-related disorders including cancer [249–251]. CR has been also demonstrated to reduce the frequency of spontaneous and inducible tumors in mice and primates [252–257]. However, CR induces significant weight loss, which has limited its efficacy and has prevented its wide-spread clinical use. In some studies, CR delayed but did not stop tumor growth progression, and some tumors including those carrying a mutation in oncogenes such as PI3K and phosphatase and tensin homolog (PTEN) have been shown to be resistant to CR [258]. In addition, CR would be predicted to be incompatible with the treatment of most cancer patients including those malnourished or at risk for severe weight loss, or affected by immunosuppression or cachexia [259]. In fact, long-term CR can impair immune functions and delay wound healing repair [260, 261]. Thus, while CR might not be compatible with cancer therapy in humans, several lessons could be learned from the molecular pathways activated by CR and/or CR mimetics.

More recently, periodic fasting has emerged as a viable strategy to protect normal cells from toxicity associated with chemotherapy, while sensitizing cancer cells to treatment. Remarkably, fasting protects normal cells from oxidants, common chemotherapeutic agents, and radiotherapy in several *in vitro* and *in vivo* models [262–264]. Specifically, glucose and serum starvation *in vitro* and a 48–72 hour food deprivation (short-term starvation or STS) in mice activated several stress-response pathways that conferred resistance to oxidative stress induced by chemotherapy. The latter effect was termed "differential stress resistance" (DSR) [265], and is based on the concept that normal cells can adapt to stressors but not cancer cells [266]. The mechanisms responsible of starvation-induced DSR in mammals include reduced circulating insulin-like growth factor 1 (IGF-1) levels and possible down-regulation of growth pathways [265]. In addition, in normal mammalian cells, starvation has been demonstrated to induce AMPK which stabilized p53 and p21 thereby resulting in cell proliferation arrest [263].

In model organisms, including bacteria and yeast, the switch from nutrient rich media to water or buffer extends longevity and also increases resistance to a variety of toxins, including ROS [262, 267, 268]. In yeast cells, the molecular mechanisms responsible for fasting-mediated protection involve the reduced activity of components of the glucose-response Ras/cAMP/PKA pathway and of the amino acids response Tor/S6K pathway [267, 269, 270]. Similarly, fasting increases resistance to oxidative stress and prolongs life-span in model organisms, such as worms and flies, by down-regulating analogous nutrient-sensing pathways such as (PI3K/AKT/TOR) [271, 272].

Cancer cells are characterized by the presence of oncogenic mutations in the same growth signalling pathways including IGF1 receptor, PI3K, PTEN, Ras, and PKA that prevent the activation of protective systems in response to starvation (DSR) [1]. In addition to the

absence of a stress resistant response to starvation, the set of mutations and chromosomal rearrangements prevent malignant cells from being able to adapt to a wide variety of conditions but particularly to those that involve broad and extreme changes such as starvation. Thus, "differential stress sensitization" (DSS) takes advantage of the fact that most mutations reduce the ability of cells to withstand changing environments. In one study, 48 to 60 hours of fasting retarded malignant tumor growth, similar to chemotherapy, and acted in synergy with chemotherapeutic drugs in killing a wide variety of cancer cells [265]. In mouse models for breast cancer, neuroblastoma, mesothelioma, and lung carcinoma, the combination of fasting and chemotherapy promoted 20–60% cancer free survival, whereas neither one of them alone resulted in any cancer free survival [263, 265]. Investigation of the molecular mechanisms underlying the STS-mediated biological effects indicate that breast cancer cells attempt to compensate for the nutrient deficiency caused by fasting by hyperactivating the AKT and mTOR/S6K pathways, increasing translation and, as a result, consuming even more energy and causing oxidation-dependent cell death [265]. A recent study in lung cancer cells, instead showed that starvation activated ATM/Chk2/p53 which sensitized cells to cisplatin treatment [263]. Many different mechanisms are likely responsible for the effects of fasting in the sensitization of different types of cancer cells. However, some of these mechanisms could to be shared by all cancer cell types.

The side effects caused by chemotherapeutic toxicity to normal cells and tissues are a major limitation of these agents. Consequently, these toxicities can limit chemotherapeutic dose intensity and compromise its overall efficacy. The need to maintain chemotherapeutic doses within this treatment range could be contributing to the survival of a sufficient number of cancer cells and lead to recurrence. Thus, reducing toxicity of chemotherapeutics by protection of normal cells and tissues, in combination with sensitizing of cancer cells, during fasting represents a promising strategy to enhance current treatments.

The safety of this intervention has been demonstrated in studies of a large cohort of patients affected by rheumatoid arthritis, who did not reveal any major adverse effect and presented clinical improvement after undergoing prolonged fasting [273]. Similarly, hypertensive patients, who fasted with only water for 10–11 days, showed normalization of systolic blood pressure values and no major side effects [274]. These studies provide a proof-of-principle for fasting in human studies.

The first clinical test of fasting in patients during chemotherapy was reported in a study where 10 patients affected by different malignancies voluntarily fasted 48–140 hours prior to and during chemotherapy. This study reported that fasting may reduce the side-effects induced by chemotherapeutic toxicity in humans [275]. Notably, these patients receiving different chemotherapeutic drugs in combination with fasting reported hunger and light-headedness as significant side-effects, and reported fewer toxicities associated with chemotherapy, including weakness, fatigue, and gastrointestinal distress [275]. Importantly, fasting did not appear to interfere with chemotherapeutic efficacy [275].

The combination of fasting with chemotherapy has gained clinical interest as a therapeutic approach for cancer patients [276]. Several clinical trials are now exploring fasting as a way to reduce the toxicity of chemotherapy and increase its efficacy in humans (e.g.

ClinicalTrials.gov numbers, NCT00757094, NCT00936364, NCT01175837, NCT01304251). Taken together, fasting is a promising therapeutic strategy for cancer patients, with the potential to be useful in combination with several chemotherapeutic drugs and across many cancer types. Future studies will determine whether fasting may potentiate the efficacy of non-toxic cancer therapies, including antibody- or immune-based strategies.

8.4 Exercise and Cancer

Growing evidence shows that chronic exercise reduces the risk of certain forms of malignancies (i.e, prevention) compared with sedentary lifestyles [277, 278]. In addition, emerging evidence indicates that chronic exercise may also be inversely correlated with cancer-recurrence as well as cancer-specific mortality (i.e., prognosis) [279, 280]. While data are not currently available from randomized trials testing the role of exercise in either prevention or prognosis, the combination of the unique metabolic features of malignant tumor cells and exercise-induced perturbations in whole-body and intracellular metabolic substrate use suggests that exercise might directly influence cancer prognosis and survival [280, 281].

Investigation into the effects of metabolic perturbations in a chemically induced rat model breast cancer showed that both CR and voluntary wheel running reduced tumor incidence and burden [282]. This reduction occurred in conjunction with reductions in circulating plasma levels of metabolic factors such as IGF-1, insulin, leptin, and increased plasma levels of IGF-binding protein 3 (IGFBP-3) and adiponectin. Further studies using the same breast cancer model showed that CR combined with voluntary wheel running inhibited tumor growth, and was associated with downregulation of mTOR-related targets including pAkt [283]. Plasma insulin and leptin were strongly correlated with intratumoral pAkt levels. In contrast, another study found tumor growth was similar between sedentary control groups and voluntary wheel running in animals orthotopically implanted with human breast cancer or prostate cancer [284, 285]. More work needs to be done in this area to determine if and how exercise effects tumor growth in these models.

Several studies in humans have investigated the effects of structured exercise training interventions on changes in metabolic parameters in subjects at-risk or with a confirmed diagnosis of cancer. For example, the Physical Activity for Total Health (PATH) Study found that structured moderate-intensity exercise training had no effect on circulating levels of IGF-1, IGFBP-3, or the ratio [286]. However, in these at-risk patients, training was associated with improvements in insulin and leptin [287]. In patients diagnosed with cancer, several studies have found that exercise training is associated with favorable changes in circulating levels of these same metabolic growth factors. For example, moderate intensity aerobic training (defined as 60–75% of baseline VO_{2peak}, 3 times per week, for 30–45 minutes per session, over 15 weeks) lead to alterations in IGF-1, IGFBP-3, and the IGF-1:IGFBP-3 ratio compared to a sedentary controls [288]. This is consistent with larger studies on aerobic exercise and resistance training in patients with breast cancer [289–291]. In another study, sedentary early-stage breast cancer patients were randomly assigned to a home-based aerobic and resistance exercise program for 16 weeks or a control group [292]. Another study using aerobic exercise found a trend toward decreased fasting insulin [293].

Indeed, the potential of exercise to alter host–tumor metabolic interactions may not only cause a shift towards a less aggressive phenotype but could also alter sensitivity to anticancer therapies. Based on current knowledge, it is difficult to predict the complex and multifaceted interaction between the host, exercise, tumor, and antineoplastic index of current therapeutics. However, exercise may affect tumor cell sensitivity in at least two ways. First, solid tumors have an abnormal vascular system that impairs effective oxygen and drug transport [294, 295], which is associated with therapeutic resistance [294, 296]. In contrast, exercise leads to favorable vascular adaptations in preclinical models of cardiac and hindlimb ischemia, as well as improvements in peripheral vascular function [297–301]. Similarly, tumors from exercised mice had significantly increased intratumoral perfusion and vascularization (i.e. physiologic angiogenesis) in orthotopic models of breast and prostate cancer [284, 285]. One study recently reported that the combination of exercise (voluntary wheel running) and chemotherapy (cyclophosphamide) was associated with significantly prolonged tumor growth delay compared with chemotherapy alone in a mouse model of murine breast cancer. Although the molecular mechanisms remain to be elucidated, hypoxia is intricately linked with tumor metabolism and exercise may effect vascularization and chemosensitivity. Second, as described above, short-term calorie restriction and/or fasting induces a stress response adaptation in normal cells that provides protection against oxidative injury from chemotherapy [262, 265]. Importantly, cancer cells have an impaired ability to respond to CR-induced stress, and may increase the therapeutic index. Exercise is also a potent form of both host and intracellular stress that may also confer similar effects to caloric restriction.

The data from these early studies, coupled with the powerful influence of exercise on metabolic homeostasis, suggest that more research in this area is needed. Specifically, investigations that adopt a translational approach are required to determine how exercise influences metabolic milieu in conjunction with biomarkers of tumor metabolic phenotypes using tissue and imaging modalities. Overall, careful elucidation of the molecular mechanisms by which exercise influences cancer metabolism could lead to rational pharmacologic agents to optimize clinical outcomes. Indeed, several lessons can be learned from interactions between cancer and fasting, cancer and CR, as well as cancer and exercise. Such research has the potential to provide new therapeutic opportunities.

8.5 Obesity and Cancer

At the other end of the metabolic spectrum, the obese phenotype is characterized by profound metabolic dysregulation, and can especially disrupt the endocrine system [302–305]. The 2007 World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) report and subsequent epidemiological studies show that obesity is associated with a number of different cancers, including colorectal, kidney, liver/gall bladder, pancreatic, esophageal, stomach, prostate, postmenopausal breast, endometrial, uterine and ovarian cancers [306]. Factors associated with weight gain and obesity, excess calorie intake/or low energy expenditure, were concluded to increase obesity-related cancers, while those protecting against weight gain were also protected against developing cancers [306]. Recent statistical modeling of data from the American Cancer Society cohort, which provided early evidence of obesity-cancer links [307], estimated that 20% of cancer

deaths are attributable to being overweight or obese [308]. Consequently, obesity and obesity-associated morbidities, such as diabetes, have been intensively investigated to determine the signaling pathways involved that lead to increased carcinogenesis [309–311] and if anti-obesogenic therapies are effective for cancer treatment and/or prevention.

Several manifestations of the obese phenotype overlap with cancer phenotypes and provide insights into approaches for metabolic modulation and possible strategies for cancer therapy. In particular, both cancer and obesity are associated with activation of cellular signaling pathways. Therefore, strategies to prevent synthesis or secretion of the signaling molecules dysregulated by obesity, or alternatively hindering their interaction with target receptors, may be a useful strategy for cancer preventative therapies. For example, therapeutic targeting of the intracellular pathways, such as JAK/STAT and PI3K/Akt/mTOR, which are often activated in both cancer and obesity, presents opportunities to intervene in carcinogenesis. Additionally, cancer and obesity activate anabolic processes, and small molecules that disrupt macromolecule biosynthesis, such as with metformin and orlistat, two drugs that lower glucose and inhibit cellular FA synthesis, respectively, might have the ability to restore homeostatic control and cellular defense systems with potential to impact cancer cells within the body [312, 313]. Indeed, metformin has received significant attention recently as a novel chemotherapeutic; for a recent review, see [314]. Finally, weight loss and reduced calorie consumption have been demonstrated to normalize altered adipokine profiles, reduce oxidative stress, and lower growth factors linking obesity and cancer, suggesting that synergistic manipulation of these pathways along with metabolism holds therapeutic promise.

In conclusion, most targets and/or compounds identified above as having potential to manipulate different aspects of cancer metabolism have not been directly tested in the context of other hallmarks. Thus, our analysis shows that more work needs to be done on cancer metabolism to identify targets or compounds that could have synergistic effects with other hallmarks against cancer. However, the emerging efficacy of physiological strategies to manipulate cancer outcomes, such as fasting/CR and exercise, and weight loss could be attributed, in part, to their ability to influence cancer metabolism as well as several other hallmarks of cancer. Future studies in this area will determine if and how dysregulated metabolism in cancer can be targeted directly or indirectly through host metabolism.

9. Tradeoffs

The recognition that cancer cells have altered metabolic pathways from their normal counterparts has opened a new opportunity to treat cancer [315]. Identification of altered metabolic enzymes and regulation of metabolism through oncogenic signals may render cancer cells highly dependent on specific pathways and metabolic programs. Mutations in some enzymes can force alternate metabolic pathways or oncogenes can drive specific metabolic programs while limiting alternatives. Targeting these pathways, therefore, may directly lead to cancer cell death or may provide opportunities to generate synthetic lethality. The challenge in targeting cancer metabolism, however, is that even if cancer cells are highly dependent on specific metabolic pathways, normal cells use these same pathways. To

what extent cancer cells and normal cells differentially rely on specific metabolic pathways will be crucial to determine the therapeutic window of any treatment [126].

A key benefit of aerobic glycolysis is the generation of metabolic intermediates for biosynthesis and cell growth [24]. Of course, normal cells also undergo rapid cell growth in development and upon stimulation. It is now evident that oncogenic signals can mimic normal growth factor signaling mechanisms. That these signals are sufficient to directly promote aerobic glycolysis has implied that normal cells also utilize these same metabolic pathways. Indeed lymphocytes transition from a quiescent metabolic state that is characterized by oxidative metabolism to one that is highly glycolytic upon stimulation with antigen in appropriate inflammatory conditions. Key pathways to drive this transition include the PI3K/Akt/mTORC1 and cMyc pathways—each of which are well established to drive aerobic glycolysis in cancer, as described above. The purpose of this glycolytic phenotype in lymphocytes is, as in cancer cells, to drive cellular growth [316]. In addition, however, aerobic glycolysis may also have a signaling role to promote expression of inflammatory cytokines.

Interestingly, not all activated lymphocytes use aerobic glycolysis. Depending on the cytokine environment, some CD4 lymphocytes develop into inflammatory effectors while others develop into suppressive regulatory T cells (Treg). The signaling pathways that drive these different CD4 T cell fates are also key metabolic regulators, and mTORC1 is essential to develop effector T cells but represses Treg [317]. Accordingly, the metabolism of these subsets differs. Effector T cells, like cancer cells with activated mTORC1, are highly glycolytic [318, 319]. Treg, however, have low mTORC1 and instead have high levels of activity in the tumor-suppressive AMPK pathway, a key inhibitor of mTORC1 and driver of oxidative metabolic pathways. Rather than favor aerobic glycolysis, therefore, Treg utilize lipid oxidation as a primary metabolic mechanism [318]. Similarly, memory T cells that return to quiescence following an immune response revert from the glycolytic phenotype of effector T cells to a lipid oxidative metabolism [320]. Suppressing lymphocyte glycolysis and promoting oxidative metabolism, therefore, inhibits the primary effector response but can enhance the generation and survival of memory T cells [321].

The metabolic implications of these distinct metabolic transitions of cells moving from quiescence to proliferation are manifold and go well beyond fundamental aspects of immunology. Firstly, lymphocytes are not unique in these transitions. Rather, it appears to be a general feature of the transition from quiescence to proliferation that cells undergo a metabolic reprogramming. Endothelial cells also undergo metabolic reprogramming [322], as do smooth muscle cells [323] and a variety of other cell types. Second, lymphocytes in the tumor environment may have difficulty competing for nutrients such as glucose. This nutrient limitation, in turn, would suppress effector lymphocytes that may play roles in anti-tumor immunity while at the same time promoting the development and activity of Treg that can maintain malignant tumors. Thirdly, efforts to target metabolism in cancer metabolism-directed therapies may have inadvertent effects to suppress normal cell proliferation. In the case of the immune system, blocking aerobic glycolysis can suppress the immune system [319, 324]. While this strategy could potentially be helpful to relieve inflammatory diseases, targeting cancer metabolism could have a tradeoff of immune suppression. In this setting,

immune suppression may increase susceptibility to infection, and inhibit the potential benefit of an anti-tumor immune response.

A key question now is not only to discover how cancer metabolism itself is programmed and functions to advance cancer, but also to understand the metabolism of normal proliferating cells. The similarities may be useful to exploit new developments in cancer metabolism to target cells in other settings. In particular, anti-inflammatory treatments may become evident from cancer metabolism drugs. Differences in the metabolic programs of normal and cancer cells will also be highly useful, as these distinctions will provide directions to target cancer metabolism, while sparing other highly proliferative cells. In some cancers, mutations will make it clear how to selectively target cancer metabolism. For most cases, however, it will be important to target cancer metabolism, while maintaining metabolic pathways regulating normal cellular function. This ongoing challenge will be critical for establishing the therapeutic window for cancer metabolism treatments as well as opening new opportunities to understand normal biology.

10. Conclusion and Future Perspectives

The energy requirements of cancer cells vary greatly from those of quiescent, terminally differentiated cells. Mammalian cells require external cues from growth factors to take up nutrients from their surroundings and in the absence of these signals they are programmed to die by apoptosis [325]. Cells must compete for limiting amounts of growth factors that direct nutrient uptake in order to survive. Under these conditions it is advantageous to maximize energy production from nutrients such as glucose, fatty acids and amino acids. This is accomplished by complete oxidation of nutrients to carbon dioxide (CO_2) and efficient coupling of ATP production though oxidative phosphorylation by the electron transport chain.

In contrast, cancer cells are characterized by growth factor-independent nutrient uptake, apoptosis evasion, and uncontrolled growth and proliferation [1]. The metabolic profile of cancer cells reflect these changes with increased nutrient uptake supplying the raw materials to synthesize the lipids, nucleotides, and proteins necessary for cell growth and proliferation [326]. The metabolic reprogramming which occurs during oncogenesis is similar to that which occurs in other cells transitioning from a quiescent to proliferative state such as induced pluripotent stem cells, embryonic stem cells and immune cells [327, 328]. This process involves a dramatic reworking of intermediary metabolism from that of a catabolic to an anabolic state. A key feature of this transition is a shift away from oxidative metabolism towards glycolytic metabolism, the net effect of which is to provide additional biosynthetic precursors for macromolecule synthesis [24]. Increased glycolytic intermediates are used to generate nucleosides, amino acids and reducing equivalents in the form of NADPH. Additionally, while levels of oxidative metabolism may be reduced, functional mitochondria are essential to cancer cell survival. In cancer cells, mitochondria function not only in energy production but also in modulation of redox status, generation of ROS, maintenance of calcium homeostasis, inhibition of apoptosis and contribute biosynthetic precursors to fuel macromolecule synthesis [247].

An important question that remains about the metabolic reprogramming which occurs in cancer is whether it is a driving force or a secondary effect of oncogenic mutations. Until recently, it was believed that metabolic reprogramming occurred secondarily to mutations in oncogenes or tumor suppressors that activate proliferation and survival signals [1]. However, a number of recent findings highlight the importance of metabolic reprogramming in driving oncogenesis (see *Oncometabolites* section), which provide strong evidence that altered metabolism is a key event that is selected for early in the oncogenic process.

Metabolic reprogramming in cancer cells is characterized by altered expression, mutation, post-translational modification, and/or changes in enzyme isoform expression. Regardless of the stage of oncogenesis during which metabolic reprogramming occurs, these unique changes could be specifically targeted to treat cancer. For these strategies to be useful, the metabolic phenotypes of several cancer types will have to be determined; techniques to do so are being developed and becoming more common [329]. Once the metabolic phenotype of a cancer is known, therapies can indirectly target upstream regulatory pathways, or directly target aberrant biosynthetic, anabolic pathways. Importantly, many cancers become resistant to radiotherapies and chemotherapy because of their altered metabolism [330]. Thus, a targeted therapy that serves to revert cancer cell metabolism back to a more catabolic state may re-sensitize a tumor to other therapies.

Much additional work is required to make targeted metabolic cancer therapies a reality. Several recent analyses, ranging from biochemical to omic-levels, have greatly increased our understanding of cancer metabolism. However, these techniques are sometimes limited to use in cell culture systems that do not account for contributions from the tumor microenvironment, the immune system, or all hallmarks of cancer. Thus, future work will be directed at measured alterations in metabolism directly in the context of other hallmarks. Together, targeting metabolism specifically or synergistically holds the potential for effectively treating a variety of cancers.

Acknowledgments

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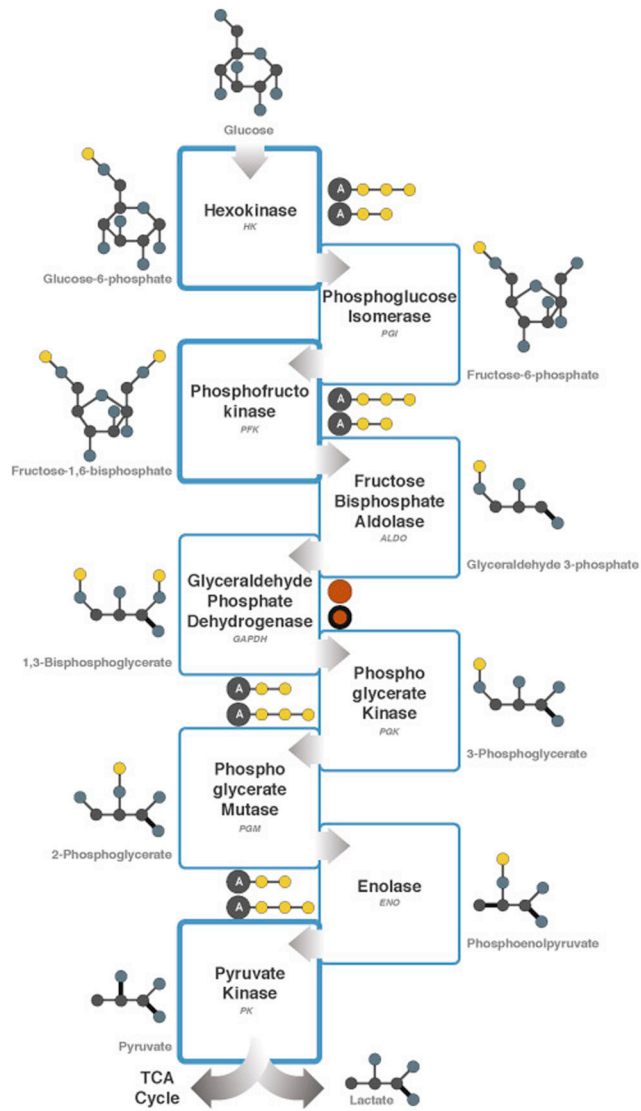
Figure 1.
Schematic of glycolysis. Enzymes altered in cancer are shown in bold. Color scheme: carbon, gray; oxygen, blue; phosphate, yellow; adenosine, A; NAD$^+$/NADH, orange; single bonds, thin; double bonds, bold.
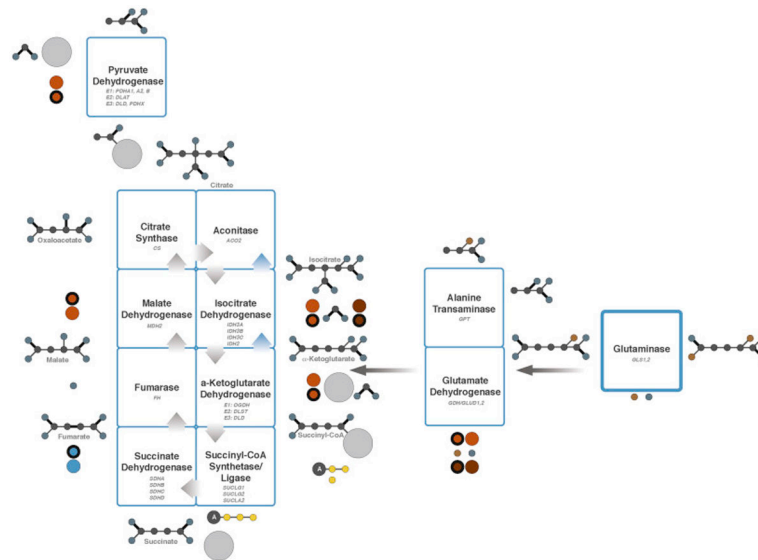
Figure 2.
Schematic of the TCA cycle and glutaminolysis. Enzymes altered in cancer are shown in bold. Reductive direction of the TCA cycle shown with blue arrows. Color scheme: carbon, gray; oxygen, blue; phosphate, yellow; nitrogen, brown; adenosine, A; coenzyme A, light gray; NAD$^+$/NADH, orange; NADP$^+$/NADPH, brown; FAD/FADH$_2$, blue; single bonds, thin; double bonds, bold.

Table 1

Prioritized pathways to target cancer metabolism

Target	Known Pathways	Predicted Manipulation
Hexokinase 2 (HK2)	Glucose Metabolism	Reduce glucose uptake and metabolism
6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3 (PFKFB3)	Glucose Metabolism	Reduce glycolysis
Pyruvate kinase isoform M2 (PKM2)	Glucose Metabolism	Activate pyruvate oxidation
Glutaminolysis	Amino Acid Metabolism	Inhibit glutamine anaplerosis
Reductive Carboxylation	Amino Acid Metabolism	Inhibit reductive carboxylation
O-GlcNAcylation	Epigenetics	Unknown
Methylation/One-carbon metabolism	Epigenetics	Unknown
Acetylation/Sirtuin deacylases (SIRTs)	Epigenetics	Unknown
Oncometabolites	Epigenetics	Inhibit oncometabolite formation and signaling
Lactate	Glucose Metabolism	Inhibit use of lactate as a fuel