Targeting Cancer Metabolism: Dietary and Pharmacologic Interventions

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ABSTRACT   Most tumors display oncogene-driven reprogramming of several metabolic pathways, which are crucial to sustain their growth and proliferation. In recent years, both dietary and pharmacologic approaches that target deregulated tumor metabolism are beginning to be considered for clinical applications. Dietary interventions exploit the ability of nutrient-restricted conditions to exert broad biological effects, protecting normal cells, organs, and systems, while sensitizing a wide variety of cancer cells to cytotoxic therapies. On the other hand, drugs targeting enzymes or metabolites of crucial metabolic pathways can be highly specific and effective, but must be matched with a responsive tumor, which might rapidly adapt. In this review, we illustrate how dietary and pharmacologic therapies differ in their effect on tumor growth, proliferation, and metabolism and discuss the available preclinical and clinical evidence in favor of or against each of them. We also indicate, when appropriate, how to optimize future investigations on metabolic therapies on the basis of tumor- and patient-related characteristics.

Significance: To our knowledge, this is the first review article that comprehensively analyzes the preclinical and preliminary clinical experimental foundations of both dietary and pharmacologic metabolic interventions in cancer therapy. Among several promising therapies, we propose treatment personalization on the basis of tumor genetics, tumor metabolism, and patient systemic metabolism. *Cancer Discov; 6(12); 1315-33. ©2016 AACR.*

INTRODUCTION

Several biological mechanisms underlying tumor initiation, progression, and metastases have been elucidated in the last decades. The discovery of oncogenes and tumor suppressor genes has revealed the biochemical bases of some hallmarks of cancer, including unrestrained proliferation, independence from growth factor stimulation, and resistance to apoptosis (1). This has paved the way for targeting crucial biochemical pathways and improving prognosis of several malignancies, including breast, lung, renal, and gastric tumors, as well as leukemias (2).

On the other hand, the impact of these discoveries has overshadowed the importance of metabolic aspects of tumor growth

for a long time. Metabolism is the set of biochemical transformations that occur within cells, including catabolic processes leading to production of energy units (in the form of ATP or reducing equivalents) and anabolic processes leading to synthesis of complex biomolecules, such as proteins, lipids, and DNA. Historically, the only metabolic pathway to be intensively studied and successfully targeted in cancer therapy is synthesis of nucleotides and deoxynucleotides (3, 4). Other key metabolic pathways have instead become the focus of intense research only recently, based on new discoveries and the efficacy plateau reached by standard anticancer treatments.

Similar to highly proliferating healthy cells, cancer cells need a constant supply of ATP and anabolic precursors to sustain crucial biochemical processes, including DNA synthesis and repair, protein and lipid synthesis, post-translational modification of proteins, membrane and organelle formation and reassembly, vesicular transport of intracellular cargos, and endocytosis. Although mitochondrial oxidation of glucose-derived pyruvate is the preferred source of ATP for proliferating healthy cells, many cancer cells divert the majority of glycolysis-derived pyruvate from mitochondrial oxidation to the synthesis of lactate (ref. 5; Fig. 1). This phenomenon, known as "aerobic glycolysis," is clinically exploited to detect increased uptake of the radiolabeled glucose analogue 18F-fluoro-2-deoxy-D-glucose (^{18}FDG) by tumors compared with normal tissues. ^{18}FDG-based positron emission tomography (PET) is

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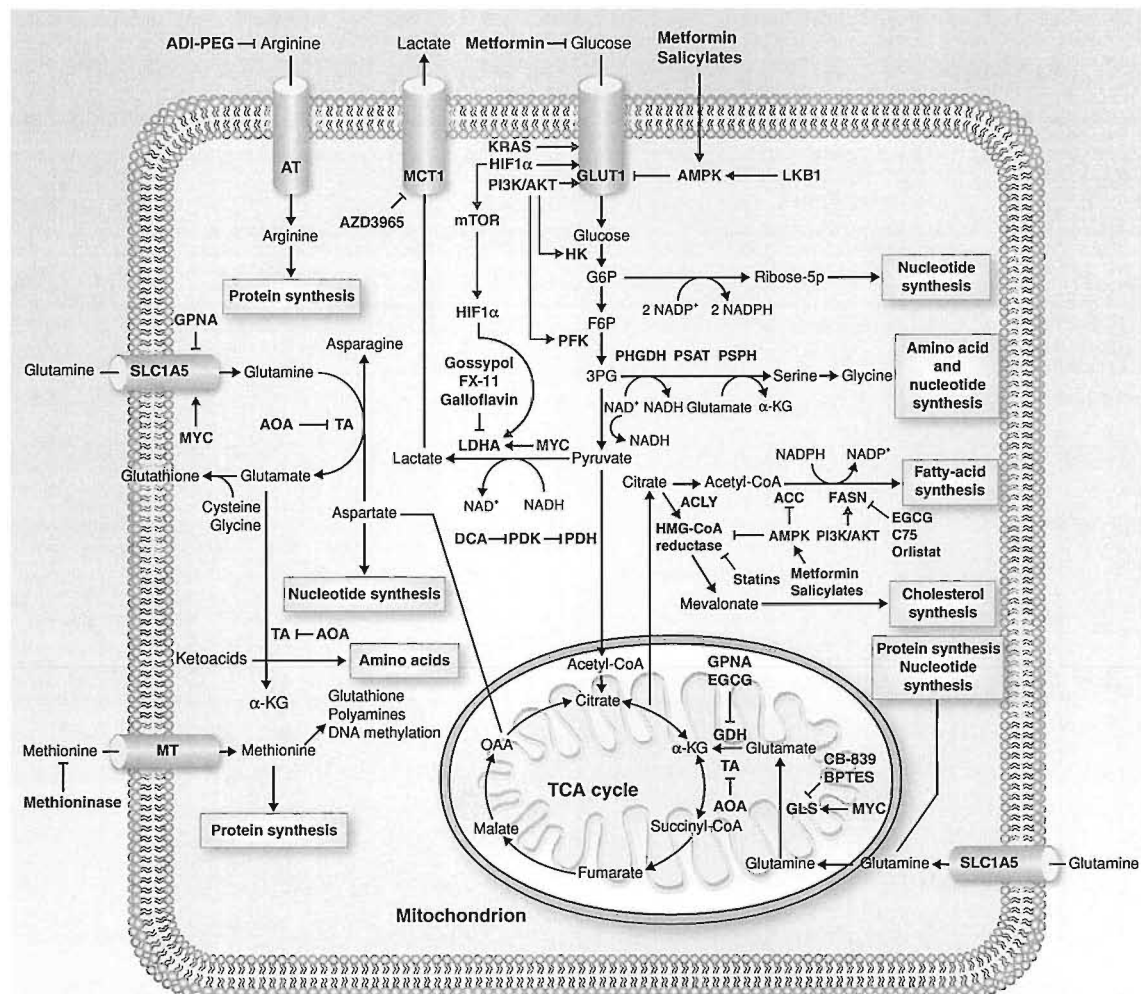
Figure 1. Main metabolic pathways deregulated in cancers and corresponding targeting drugs. Cancer cells upregulate both catabolic and anabolic pathways to optimize energy and macromolecule production. Glucose and glutamine are central biomolecules that provide cancer cells with most of the energy and metabolites required for growth and proliferation. Glucose is uptaken by tumor cells through the GLUT1 transporter and enters glycolysis. The glycolytic intermediate G6P can be diverted to the pentose phosphate pathway to form ribose-5P (nucleotide synthesis) and reducing equivalents in the form of NADPH (anabolic processes). Another glycolytic intermediate, 3-PG, can be diverted to synthesis of serine and glycine, which can be incorporated into proteins or nucleotides, or used as precursors of other biomolecules. Finally, glucose-derived pyruvate can be converted to lactate by LDHA, oxidized in the mitochondrial TCA cycle or converted to citrate to fuel synthesis of FAs and cholesterol. Synthesis of FAs requires ACC and FASN enzymes, whereas HMG-CoA reductase is the key enzyme for cholesterol synthesis. Glutamine enters tumor cells through the SLC1A5 transporter and is used for protein or nucleotide synthesis or can be converted to glutamate and then α-KG, which can be either oxidized in the mitochondrial TCA cycle or undergo reductive metabolism to form citrate, thus contributing to FA and cholesterol synthesis. Cytoplasmic glutamine can also be transaminated to form amino acids from corresponding ketoacids. Arginine and methionine are uptaken from the external environment through specific transporters and then used for protein synthesis or other purposes. ACC, acetyl-CoA carboxylase; ACLY, ATP citrate lyase; ADI-PEG, PEGylated arginine deiminase; AMPK, AMP-activated protein kinase; AOA, aminooxyacetate; AT, arginine transporter; DCA, dichloroacetate; FASN, fatty acid synthase; F6P, fructose 6 phosphate; GDH, glutamate dehydrogenase; GLS, glutaminase; GLUT1, glucose transporter 1; G6P, glucose-6 phosphate; HIF1α, hypoxia-inducible factor 1α; HK, hexokinase; LDHA, lactate dehydrogenase A; LKB1, liver kinase B1; MT, methionine transporter; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; OAA, oxaloacetate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFK, phosphofructokinase; PHGDH, phosphoglycerate dehydrogenase; PI3K, phosphatidylinositol 3-kinase; PSAT, phosphoserine aminotransferase; PSPH, phosphoserine phosphatase; ribose 5-P, ribose 5-phosphate; TA, transaminase; TCA, tricarboxylic acid; α-KG, α-ketoglutarate; 3-PG, 3-phosphoglycerate.

actually used for diagnostic and staging purposes, and also to monitor tumor response to therapies (6). Recent studies have shown that not only glycolysis but many other metabolic pathways can be deregulated in tumor cells. Moreover, intimate connections between oncogenes/tumor suppressor genes and metabolic reprogramming suggest that deregulated metabolism crucially contributes to unrestrained proliferation of cancers (5, 7). Given the large metabolic differences between healthy and neoplastic cells, there is a hope to selectively target tumor metabolism, while limiting toxicities to normal tissues.

Because cancer cell metabolism is complex and potentially heterogeneous within big tumor masses, recent technical advancements, including molecular magnetic resonance spectroscopy (MRS) and hyperpolarized MRI, could offer a detailed picture of the utilization/production of several metabolites by in vivo human tumors in different disease localizations, thus accounting for metabolic heterogeneity. These techniques could also be useful to follow dynamic evolution of tumor metabolism during disease progression or in response to therapies (8, 9).

In the first part of this review, we analyze the main metabolic cascades that are deregulated in tumors. A clear understanding of these pathways and their role in tumor cell proliferation and survival is essential to identify targets for effective therapies. In the second and third parts of the article, we respectively review the dietary and pharmacologic strategies that hold promise to successfully target tumor metabolism on the basis of available preclinical evidence.

METABOLIC PATHWAYS THAT SUSTAIN CANCER CELL SURVIVAL AND PROLIFERATION

Glucose

Upregulated aerobic glycolysis provides several benefits to cancer cells (5). First, at physiologic blood concentrations, glucose ensures sufficiently fast ATP production to fulfill energetic demands, while contemporaneously fueling anabolic processes through biomass production; second, pyruvate-derived lactate, when excreted, creates an extracellular acid environment that recruits macrophages and other immune cells, thus favoring metastasis; third, pyruvate can be used to produce oxaloacetate and the amino acids (AA) alanine and aspartate, which take part in the synthesis of proteins or other biomolecules (5, 10). In summary, aerobic glycolysis can fulfill most of the energetic and metabolic needs of highly proliferating cancer cells, including AA biosynthesis when a proper source of nitrogen groups (usually deriving from glutamine) is also provided. In tumors, aerobic glycolysis is often stimulated by oncogenes, including PI3K and RAS, which induce expression of the glucose transporter gene GLUT1 and of the glycolytic enzymes hexokinase and phosphofructokinase, and contemporaneously inhibit mitochondrial oxidation of pyruvate (Fig. 1).

Recent in vivo studies on tumors showed that part of glucose-derived pyruvate can be diverted into the mitochondrial tricarboxylic acid (TCA) cycle to produce additional energy or intermediates for synthesis of fatty acids (FA) or other nonessential AAs, such as glutamate and glutamine (11, 12). While reducing the dominance of aerobic glycolysis as the primary source of energy and anabolic precursors for rapidly

proliferating tumor cells, these studies confirm glucose as the major metabolic substrate for in vivo malignancies.

Preclinical evidence suggests that targeting deregulated glucose metabolism is a potentially effective anticancer approach. Indeed, reducing extracellular glucose or inhibiting glycolysis through 2-deoxy-D-glucose induces proliferation arrest in several cancer cell lines and also synergizes with cytotoxic treatments to activate apoptosis (13, 14); these effects are especially strong in cells with compromised mitochondrial oxidative phosphorylation (15). Inhibiting lactate production by the lactate dehydrogenase A (LDHA) enzyme is another way to halt glycolysis progression by preventing NAD^+ regeneration from NADH, which is toxic to highly glycolytic cancer cells (16). Moreover, dietary regimens that reduce glycemia also enhance the antitumor activity of chemotherapy and prolong survival of mice xenografted with human tumor cells (13). Finally, the hyperglycemic/diabetic state is associated with worse prognosis in patients with glioblastoma multiforme (GBM), colorectal cancer, or acute leukemia (17–20).

Two different approaches can be exploited to target aerobic glycolysis in cancer therapy: reducing blood glycemia (systemic approach) or inhibiting specific enzymes in the glycolytic cascade (cell-autonomous approach). The former strategy requires a careful selection of patient subgroups that, based on their glycemic state (hyperglycemic vs. euglycemic), tumor avidity for glucose as detected through ^{18}FDG-PET, or molecular tumor profile (e.g., RAS or PI3K activation), are more likely to benefit from it. In the cell-autonomous approach, the most suitable molecular targets need to be identified, and potent and selective inhibitors to be synthesized.

Amino Acids

Unrestrained tumor proliferation requires continuous replenishment of AAs to be used as building blocks for structural and enzymatic proteins, as precursors of essential biochemical components, including FAs, other AAs, nucleotides and the antioxidant glutathione, or, finally, as monocarbon unit donors. Similar to normal tissues, cancer cells are usually able to synthesize some AAs (referred to as "nonessential" AAs), whereas they rely on the blood supply of the remaining, "essential" AAs. However, some tumors may lose the ability to synthesize one specific nonessential AA, thus becoming dependent on the external supply. This phenomenon is known as "auxotrophy."

One potential strategy to target AA metabolism in tumors is to deplete circulating blood AAs through dietary or pharmacologic interventions. However, because subtracting essential AAs might prove toxic to normal tissues, this strategy is reasonably exploitable only for auxotrophic tumors. One alternative strategy consists of targeting intracellular enzymes involved in the metabolism of specific AAs, without affecting their blood concentration.

Glutamine is a nonessential AA that can be obtained from dietary sources, protein degradation in muscle cells, or de novo synthesis. Several types of cancer cell lines, including some derived from breast and lung tumors, rely on glutamine supply to survive and proliferate (21, 22). Cancer cells can directly internalize glutamine from the extracellular environment (e.g., the blood) through the glutamine transporter SLC1A5 located on the plasma membrane (21). Alternatively,

glutamine, as well as other AAs, can be derived from lysosomal degradation of extracellular proteins that are internalized through macropinocytosis (23). This latter mechanism has been described in *RAS*-mutated pancreatic and bladder cancers, which depend on extracellular glutamine, but become glutamine-independent if provided with sufficient amounts of extracellular albumin, as well as other proteins to be degraded in lysosomes (23).

In tumors, glutamine takes part in synthesis of proteins or nucleotides (24), stimulation of glucose uptake (25), and activation of the mammalian target of rapamycin (mTOR; refs. 26, 27). However, the majority of intracellular glutamine is converted by the glutaminase (GLS) enzyme to glutamate, which is used as a precursor of glutathione or to produce alpha-ketoglutarate (α-KG) through reactions catalyzed either by the enzyme glutamate dehydrogenase (GDH), or by transaminases (TA; refs. 24, 27–30; Fig. 1). Glutamine-derived α-KG can undergo either oxidative metabolism in mitochondrial TCA cycle (28) or reductive metabolism to form isocitrate and citrate in the "reverse TCA cycle," thus contributing to FA and cholesterol synthesis (refs. 24, 29, 30; Fig. 1). The oncogenic protein MYC directly stimulates catabolic metabolism of glutamine by inducing expression of both glutamine transporter genes *SLC1A5* and *GLS* (31); of note, GLS inhibition and glutamine deprivation halt proliferation of MYC-overexpressing tumor cells (30, 31). A recent paper has also shown increased oxidative metabolism of glutamine in *PIK3CA*-mutated, but not *PIK3CA*-wild-type (WT), colorectal cancers (32). On the other hand, different studies suggest that glutamine uptake may be nonessential for *in vivo* GBM and lung tumors, in which glucose utilization is likely sufficient to satisfy both energetic and anaplerotic needs (11, 12, 33). Because glutamine intracellular utilization can significantly differ between *in vitro*-grown cancer cells and tumors that are embedded in their original microenvironment, tumor dependency should be assessed by measuring glutamine uptake and utilization in *in vivo* tumors, e.g., through nuclear magnetic resonance spectroscopy that tracks the fate of intravenously administered radiolabeled glutamine (12).

Because it is difficult to reproducibly modify glutamine blood concentration through dietary interventions, pharmacologic strategies targeting glutamine transporters or glutamine-metabolizing enzymes seem to be the most promising approaches to target glutamine-addicted tumors.

Methionine is an essential AA taking part in protein synthesis, DNA and protein methylation, and synthesis of glutathione and polyamines (34). Normal cells cannot synthesize methionine from other AAs, but they can produce it from homocysteine. On the contrary, several tumor cell lines, including colon, breast, and prostate, cannot proliferate and survive in the absence of methionine (35). Methionine contributes to activating oncogenic pathways in GBM, and methionine deprivation negatively affects tumor cell proliferation (36). Coherent with *in vitro* data, lowering dietary intake of methionine inhibits tumor growth in *in vivo* mouse and rat models, and also reduces tumor size synergistically with cytotoxic treatments (37). Dietary restriction of methionine is therefore a promising anticancer approach that deserves further investigation in future studies.

Arginine is a nonessential AA, used for protein synthesis or as a precursor of nitric oxide, polyamines, creatine, as well as glutamine and proline when these AAs are scarce (38). Moreover, arginine is involved in mTOR activation (26) and growth hormone (GH), insulin, and insulin-like growth factor 1 (IGF1) secretion, especially after strenuous physical exercise (39). Sources of circulating arginine include diet, protein degradation, and *de novo* synthesis, which is initiated by the argininosuccinate synthetase 1 (ASS1) enzyme (40). Its dietary uptake becomes essential only in conditions of increased tissue growth (e.g., during childhood) or under specific stresses (such as inflammation; ref. 41).

Several tumors, including some melanomas, hepatocellular carcinomas (HCC), and mesotheliomas, epigenetically repress *ASS1* expression through methylation of its promoter (42). These cancers depend on arginine uptake from the extracellular environment (the blood and/or nearby normal or tumor cells) to survive and proliferate (43). Despite causing tumor dependency on extracellular arginine, *ASS1* inactivation confers specific metabolic advantages, including glutamine independence or aspartate-mediated enhancement of pyrimidine nucleotide production (44, 45). Reducing arginine availability in *ASS1*-repressing tumors holds promise as a potent and selective anticancer strategy.

Serine and glycine are nonessential AAs, which can be synthesized from the glycolytic intermediate 3-phosphoglycerate through a biochemical cascade initiated by the phosphoglycerate dehydrogenase (PHGDH) enzyme (Fig. 1). They take part in redox balance and *de novo* synthesis of purines and glutathione, thus contributing to protein, DNA, and lipid synthesis (46). Intracellular serine also stimulates cell proliferation through mTOR activation (47). Due to these multiple functions, highly proliferating cancer cells need continuous replenishment of both serine and glycine.

Some tumors uptake serine from the external environment (48), and could be sensitive to dietary restriction of serine-containing foods. Despite the lack of data in human subjects, experiments on mice demonstrate that limiting dietary intake of serine is safe and capable of lowering serine and glycine blood levels by about 50% and delaying tumor development (49). Moreover, serine restriction synergizes with metformin to inhibit growth of already-established cancers (50). Other malignancies, especially triple-negative breast cancers (TNBC) and melanomas, overexpress serine synthesis enzymes, especially PHGDH, through amplification of the *PHGDH* gene, thus becoming completely independent from external serine supply (51, 52). Suppressing PHGDH inhibits proliferation of PHGDH-overexpressing tumors and holds promise to target highly aggressive malignancies (48). The price paid by PGHDH-overexpressing cells for their serine independence is the depletion of glycolytic intermediates. As a consequence, glucose deprivation or metformin-induced inhibition of mitochondrial oxidative phosphorylation could prove especially toxic to cancer cells relying on glucose-dependent synthesis of serine (Fig. 1; ref. 50).

Lipids

Lipids are essential components of cell membranes, contributing to their fluidity and to the activation of membrane-anchored signal transduction enzymes. Cancer cells depend

on continuous replenishment of FAs to form new membranes and organelles. However, although most normal cells internalize dietary or fatty tissue-derived FAs that circulate in the bloodstream either as free FAs or as part of lipoproteins, most cancer cells *de novo* synthetize their FAs independently from nutrient availability and hormone stimulation (53). FA synthesis begins with the conversion of citrate to acetyl-CoA and then acetoacetyl-CoA, which is finally elongated to form palmitate and other FAs. Crucial enzymes in this process are acetyl-CoA carboxylase (ACC), which catalyzes the limiting-step reaction of the cascade, and the multisubunit FA synthase (FASN) enzyme (Fig. 1). Major sources of citrate to be used for FA synthesis are glucose (5) and glutamine-derived α-KG, especially under hypoxia or disruption of the mitochondrial oxidative machinery (24, 29, 30).

Crucially, FASN is overexpressed by most tumors, including breast, ovarian, lung, colon, endometrial, gastric, and head and neck cancers (53, 54). Of note, MAPK/PI3K oncogenic pathways and FASN can activate each other through a positive feedback loop that couples cellular proliferation with metabolic processes (55). Moreover, FASN protein levels are associated with worse prognosis in human cancers (56, 57). Finally, pharmacologic inhibition of FASN with cerulenin, C75, the antiobesity drug orlistat, and green tea polyphenols, such as epigallocatechin-3-gallate (EGCG), results in significant *in vitro* and *in vivo* anticancer effects, which can be rescued by high FA extracellular concentrations (58, 59).

Targeting FA synthesis either indirectly (e.g., by inhibiting glycolysis/glutamine metabolism) or directly (by inhibiting ACC/FASN) holds promise to selectively affect FA metabolism in tumor cells. However, recent data suggest that some tumors can uptake extracellular lipids, especially lysophospholipids, that are either present in the bloodstream or produced by nearby cells in the tumor microenvironment (60, 61). From a therapeutic perspective, this implies that inhibition of *de novo* FA synthesis should be combined with inhibition of extracellular lipid uptake to fully deplete tumor intracellular lipids.

Cholesterol

Cholesterol is another essential component of biological membranes and precursor of isoprenoids and steroid hormones. It is synthesized from acetyl-CoA through a series of biochemical reactions, whose first steps involve the condensation of three acetyl-CoA molecules to form 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). The rate-limiting step reaction of the cascade consists in the formation of mevalonate from HMG-CoA, which is catalyzed by the HMG-CoA reductase (HMGCR) enzyme. Interestingly, HMGCR is overexpressed in several tumors, leading to increased production of cholesterol and isoprenoids from glucose- or glutamine-derived acetyl-CoA (62). Moreover, HMGCR inhibition by statins (e.g., simvastatin or atorvastatin) halts proliferation or induces apoptosis in several tumor cell lines synergistically with chemotherapeutic agents (62–64). Targeting cholesterol metabolism is therefore a promising research topic in the field of tumor metabolism.

Ketone Bodies

Under hypoglycemia or reduced glucose uptake, hepatocytes convert excess acetyl-CoA derived from FA β-oxidation to ketone bodies, especially acetoacetate and β-hydroxybutyrate, which are released into the bloodstream and used by peripheral cells to produce acetyl-CoA and fulfill energetic and biosynthetic requirements. FA-derived ketone bodies are therefore essential for normal tissues under conditions of glucose scarcity.

Different from healthy cells, most cancer cells cannot utilize ketone bodies as their primary energy source, mainly because they do not usually express enzymes that convert ketones to acetyl-CoA, and also because they are addicted to glucose for their energetic and biosynthetic needs (5, 65). Moreover, by forcing tumor cells to oxidize acetyl-CoA in mitochondria, ketone bodies may delay glycolysis progression and prove toxic to highly glycolytic tumors (66). Coherent with *in vitro* data, *in vivo* studies using mouse models have demonstrated reduced tumor growth and increased animal survival after increasing circulating ketone body concentration through specific dietary interventions (67, 68).

On the basis of preclinical evidence, increasing ketone bodies in the blood could synergize with glucose reduction or pharmacologic glycolysis inhibition.
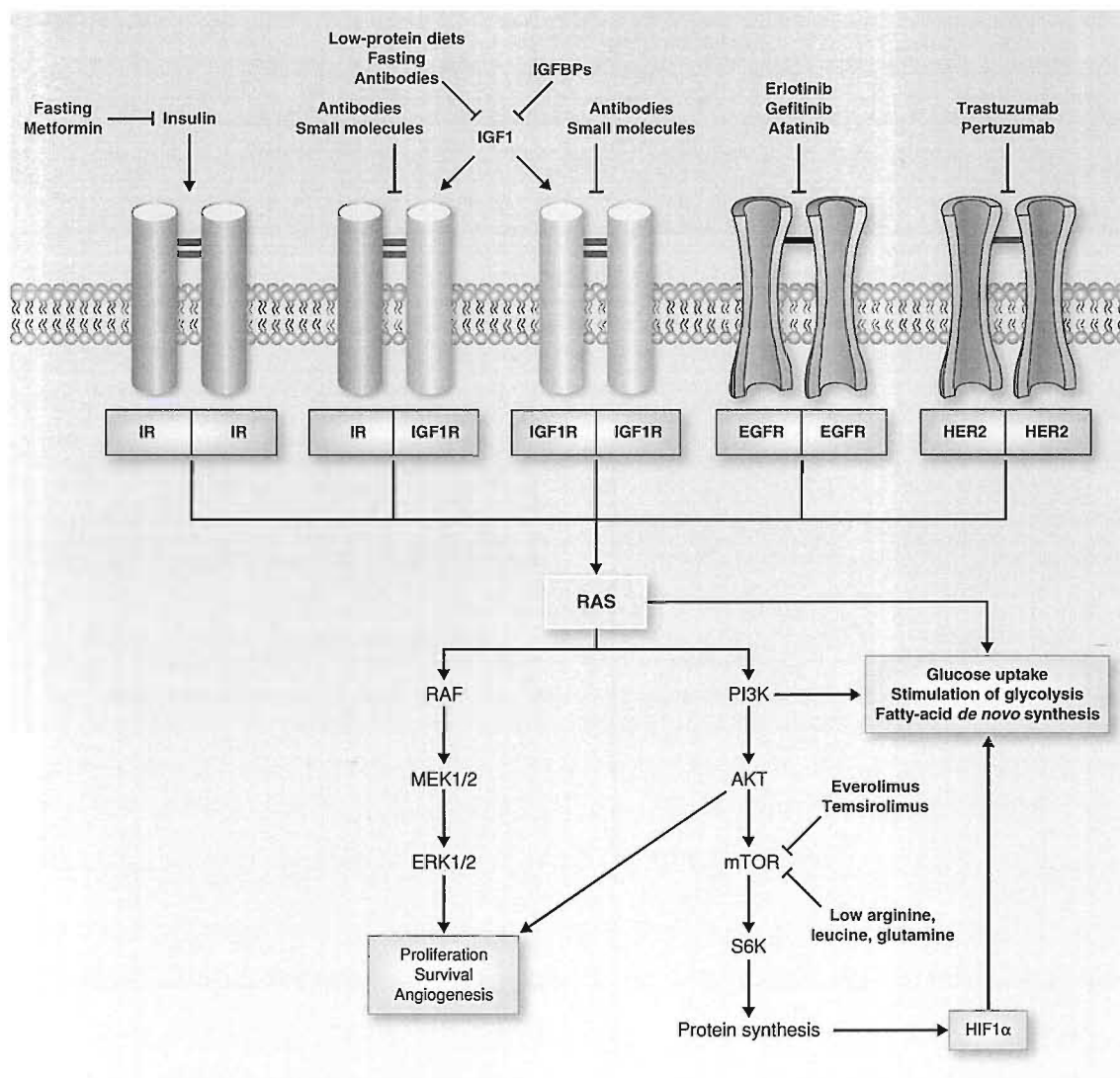
Insulin

Insulin is an essential pancreatic hormone that regulates carbohydrate and lipid metabolism by stimulating glucose uptake in peripheral cells and FA synthesis in the liver. It binds to insulin receptor (IR), which in turn activates the IR-stimulated/RAS/RAF/MEK/MAPK and RAS/PI3K/AKT/mTOR signal transduction cascades (ref. 69; Fig. 2).

When added to cell growth media, physiologic insulin concentrations stimulate cancer cell proliferation (70). Moreover, exogenous insulin and hyperinsulinemia accelerate tumor growth and metastases in animals, whereas IR inhibition reverses these effects (71, 72). Mechanisms responsible for insulin-mediated oncogenic activities include: (i) direct activation of IR and its downstream MAPK and PI3K/AKT signal transduction pathways (69); (ii) insulin-stimulated IGF1 production by hepatocytes; (iii) direct activation of the IGF1 receptor (IGF1R) by insulin. Retrospective studies suggest that hyperinsulinemic patients are more prone to develop aggressive tumors and to die of cancer (73, 74). However, it is presently unknown whether reducing blood insulin levels can affect already-established tumors. Moreover, although blood insulin can be reduced through dietary (e.g., carbohydrate-restricted regimens) or pharmacologic (e.g., metformin) approaches in hyperglycemic/hyperinsulinemic patients, euglycemic subjects would hardly benefit from this strategy because of the risk of causing insulin-dependent diabetes, thus increasing blood glucose and possibly overfeeding tumor cells.

IGF1

The peptide hormone IGF1 is produced by hepatocytes and other cell types when stimulated by GH, insulin (69), and protein-rich diets (75). IGF1 plays its major physiologic role during adolescence, when it promotes growth of several tissues, including bone cartilage (76). A significant fraction of circulating IGF1 is bound to IGF1 binding proteins (IGFBP), especially IGFBP1, IGFBP2, and IGFBP3, which sequester IGF1 and reduce its availability to target tissues (76).

Interestingly, chronically high blood IGF1 levels are associated with increased tumor risk and worse cancer prognosis (73, 77, 78). IGF1 binds to and activates IGF1 tyrosine kinase (TK)

Figure 2. Connections between insulin/insulin-like growth factor 1 signaling and metabolic pathways in tumor cells. Insulin receptor (IR) and IGF1R can either homo- or heterodimerize to activate their TK domains; this stimulates downstream RAS/RAF/MEK/ERK and RAS/PI3K/AKT/mTOR signal transduction pathways, which induce survival, proliferation, angiogenesis, and ribosomal synthesis of several proteins, including HIF1α. PI3K, RAS, and HIF1α promote crucial metabolic modifications in neoplastic cells, including glucose uptake and aerobic glycolysis, as well as *de novo* synthesis of fatty acids. Because RAS/RAF/MEK/ERK and RAS/PI3K/AKT/mTOR cascades can also be activated by other membrane receptors, including EGFR and HER2, combining inhibition of the IGF1/IGF1R pathway with targeting of other TK receptors or their downstream mediators (e.g., mTOR) could synergistically inhibit cancer cell proliferation and survival. EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HIF1α, hypoxia-inducible factor-1α; IGF1, insulin-like growth factor 1; IGFBP, IGF1 binding protein; IGF1R, IGF1 receptor; IR, insulin receptor; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; S6K, S6 kinase.

receptors (namely, IGF1R and IGF2R) on tumor cells, thus activating phosphorylation cascades that converge on the RAS/ RAF/MEK/MAPK or RAS/PI3K/AKT/mTOR signaling pathways, which stimulate cell proliferation and inhibit apoptosis (69). Targeting IGF1 signaling could affect tumor growth while contemporaneously hampering IGF1-induced immunodepression and stimulating an effective antitumor response (79, 80).

Disappointingly, studies conducted so far in patients with metastatic breast, pancreatic, or lung cancer failed to demonstrate the efficacy of IGF1/IGF1R axis inhibitors (81, 82). Better patient selection on the basis of circulating IGF1 levels or activity of biochemical cascades downstream of IGF1R may improve effectiveness of IGF1R inhibition. Moreover, concomitant targeting of kinases that act in parallel with

(e.g., EGFR or HER2) or downstream (e.g., mTOR) of IGF1R to activate the same transduction pathways may synergize with IGF1/IGF1R inhibitors (refs. 83, 84; Fig. 2). Finally, dietary/pharmacologic interventions that reduce hyperglycemia that can be induced by IGF1 axis inhibition could significantly improve the efficacy of IGF1R inhibitors (82).

Deregulation of TCA Cycle Enzymes in Human Cancers

Among emerging cancer-specific metabolic alterations, deregulation of different TCA cycle enzymes is interesting based on its potential therapeutic implications. Inactivation of succinate dehydrogenase in human paragangliomas, or fumarate hydratase in aggressive papillary kidney carcinomas, leads to accumulation of succinate and fumarate, respectively, which induce stabilization of hypoxia-inducible factor-1α (HIF1α) and stimulate glycolytic metabolism (85, 86). Moreover, the TCA cycle isocitrate dehydrogenase (IDH) enzyme, which catalyzes the reversible conversion of isocitrate to α-KG, is frequently mutated in most low-grade astrocytomas and secondary glioblastomas, but also in acute myeloid leukemias, chondrosarcomas, and cholangiocarcinomas (87). *IDH1* mutations lead to a change of IDH1 enzymatic activity and to a significantly increased production of the α-KG metabolite 2-hydroxyglutarate (2-HG), which regulates DNA methylation and contributes to mTOR activation (88). Of note, two-dimensional correlation MRS has been used to detect *in vivo* 2-HG in patients with glioma harboring *IDH1* mutations; in the future, this technique could be used as a noninvasive diagnostic tool and to study dynamic 2-HG changes during tumor progression or response to therapy (89).

Investigating how TCA cycle enzyme deregulation affects tumor growth will be crucial to design effective antitumor strategies, including IDH inhibitors or tyrosine kinase inhibitors (TKI; refs. 87, 90).

DIETARY INTERVENTIONS

Dietary changes bear the potential to affect tumor growth by modifying the blood concentration of many biomolecules and metabolites that sustain cancer cell proliferation. However, given the variety of possible approaches and their complex and sometimes unpredictable impact on the multiplicity of blood metabolites, it is not a trivial aim to identify the ideal anticancer diet. Based on preclinical studies, this diet should target most deregulated metabolic pathways of tumor cells, without significantly affecting healthy tissues. In this overview, we will focus on those approaches that have a solid preclinical evidence of safety and antitumor activity, as summarized in Table 1. Following the observation that a moderate, chronic caloric restriction significantly reduces cancer incidence in nonhuman primates (91, 92) and other mammalian species (93), many currently investigated antitumor dietary approaches are based on the concept that restricting/modulating nutrient intake can reduce metabolite delivery to tumor cells.

Fasting

Avoiding calorie intake for an average of 3 to 5 consecutive days, also referred to as "short-term fasting" or STF, induces multiple systemic metabolic changes that could hamper cancer growth, including: (i) reduction of blood glycemia; (ii) decreased insulinemia due to peripheral insulin sensitization; (iii) reduction of IGF1 and increased IGFBP levels; and (iv) increased blood ketone bodies (94, 95). Although relative glycemia reduction depends on baseline glucose concentration and can be lacking in euglycemic subjects, IGF1 and IGFBPs are more consistently reduced (by about 50%) and increased (by 5-fold), respectively, during STF (96, 97). Fasting can therefore affect both tumor metabolism and circulating growth factors. However, its effects on circulating AAs are still unclear. In healthy volunteers, fasting for 4 to 5 days induced a moderate increase in methionine, valine, leucine, and isoleucine

Table 1. Main characteristics of dietary interventions under clinical investigation

Dietary intervention	Metabolic effects	Potential clinical effects	Risks and limitations	Ongoing trials
Short-term fasting	– Reduced glycemia – Reduced insulinemia – Reduced IGF1 – Increased ketones	– Reduced side effects from CT – Inhibition of tumor cell proliferation – Apoptosis induction – Synergistic with CT	– Poor tolerability – Weight loss – Increased amino acid concentration	– Metastatic prostate cancer: NCT02710721 – Combined with cisplatin and gemcitabine: NCT00936364
Ketogenic diets	– Reduced glycemia – Reduced insulinemia – Increased ketones – Increased IGF1 (if hyperproteic)	– Inhibition of tumor cell proliferation – Apoptosis induction – Synergistic with CT	– Poor palatability	– GBM: NCT02302235; NCT02046187; NCT01865162; NCT01754350;
FMD	– Reduced glycemia – Reduced insulinemia – Reduced IGF1 – Increased ketones	– Reduced side effects from CT – Apoptosis induction – Inhibition of tumor cell proliferation – Synergistic with CT	– Poor palatability	– HER2-negative breast tumors (neoadjuvant): NCT02126449

Abbreviations: CT, chemotherapy; FMD, fasting-mimicking diet.

blood concentrations, while poorly affecting or mildly reducing other AAs (98). However, due to significant differences in systemic AA metabolism between healthy individuals and patients with cancer, these results cannot be directly translated to the population of subjects with advanced tumors.

In *in vitro* experiments, STF is mimicked by short-term starvation (STS), consisting of the reduction of glucose and growth factors in cell growth media. STS sensitizes several cancer cell lines to different chemotherapeutic drugs, while relatively protecting normal cells (13, 79, 96). This differential stress response (DSR) may depend on the ability of nutrient-restricted healthy cells to halt proliferation and anabolic processes, and to activate catabolic processes (such as autophagy) and protective functions, such as DNA and protein repair, which preserve genome and proteome integrity during the resting state. In contrast, most cancer cells are unable to halt proliferation and anabolism, even in conditions of nutrient scarcity; this exposes them to rapid ATP depletion and increased DNA damage by several chemotherapeutic agents, including alkylating agents and topoisomerase inhibitors (99). However, tumor cell sensitivity to nutrient and growth factor deprivation is not a general rule, because certain tumors with a constitutively active PI3K/AKT pathway grow independently from IGF1 and insulin stimulation and are therefore insensitive to both *in vitro* starvation and *in vivo* caloric restriction (100). Understanding whether and how oncogenic pathways other than PI3K/AKT influence tumor response to starvation is of crucial importance to better select patient candidates to STF.

In *in vivo* mouse models of several tumors, STF inhibits cancer growth similarly to chemotherapy (13). Moreover, fasting reduces chemotherapy-induced toxicities to several normal tissues and synergizes with chemotherapy or radiotherapy to kill tumor cells (13, 96). These effects translate into significant survival prolongation in animals fasting during chemotherapy. The DSR between normal and cancer tissues therefore creates a therapeutic window to specifically target cancer cells, while sparing normal tissues.

Recently, STS and STF have also shown synergistic *in vitro* and *in vivo* antitumor effects with a wide range of TKIs that inhibit growth of lung, breast, and colorectal human cancers (101). The observed synergy can be explained through the ability of STS to potentiate TKI-induced inhibition of growth signaling cascades, including the MAPK and PI3K/AKT pathways, thus preventing rebound oncogene activation that occurs upon exposure to single-TKI treatment (102). Although preliminary, these pieces of evidence hold promise to extend the possible combinatorial applications of fasting to most clinical settings.

Ongoing trials are investigating the safety and efficacy of STF in advanced human cancers. Preliminary data indicate that STF is safe and potentially useful to reduce chemotherapy-induced side effects in humans with several types of tumors, without apparently compromising treatment efficacy (103). Patients lose as much as 10% of their weight during STF, but weight loss is usually reversible. In one recent study, patients with stage II/III breast cancer undergoing STF during (neo)adjuvant chemotherapy reported less hematologic toxicities and less chemotherapy-induced DNA damage to circulating blood cells, including lymphocytes, compared with patients following a regular diet (104).

The potential impact of fasting on the balance between inhibitory and antitumor immune cell populations is another field of intensive research due to its possible dramatic consequences on the outcome of new immunotherapies in the treatment of several cancers. Promising in this respect are the preliminary findings that fasting reduces chemotherapy-induced lymphopenia in mice and humans (105) and that fasting-mediated antitumor effects depend on the presence T lymphocytes in mice (106).

Ketogenic Diets

Commonly recommended diets ensure a daily caloric intake of 1,500 to 2,000 Kcal, with a net predominance of carbohydrates over proteins and fats (carbohydrates:proteins:fats ratio of around 60:20:20). On the other hand, diets rich in fats and poor in simple and complex carbohydrates force the organism to switch from carbohydrate to FA metabolism and are referred to as "ketogenic diets" (KD), because increased FA oxidation raises blood levels of ketone bodies. Depending on the specific ratio of macronutrient composition (fats:proteins:carbohydrates), origin of fats (animal versus vegetal), and total calorie content, different KDs can produce quite different metabolic effects (107).

Based on the ability of normal, but not tumor, brain cells to use ketone bodies as their primary energy source, KDs have been first proposed to treat brain malignancies (65, 108). The first reported study dates back to 1995, when two young children with grade III and IV astrocytomas recurring after extensive chemotherapy and radiotherapy treatment were given a specific KD, and both experienced reduced glucose uptake by the tumor and durable tumor control (109). Since then, several small studies have reported good tolerability and potential clinical activity of KDs in advanced tumors. In one study, 10 subjects with different metastatic cancers progressing on standard therapies and a positive [18]FDG-PET scan were fed a KD with as little as 5% carbohydrate content for 28 days, while protein and fat ingestion was encouraged to maintain a stable total caloric intake. The dietary intervention was well tolerated and increased β-hydroxybutyrate blood concentration, while reducing glucose and insulin levels. Interestingly, some patients remained stable at 1-month [18]FDG-PET reevaluation, and stability correlated with increased plasma ketones (110). In another study, 16 subjects with different end-stage cancers limited carbohydrate daily intake to less than 70 g and were given meals rich in proteins and oils. This diet was globally quite well tolerated and 5 subjects with higher ketone body urinary excretion had stable disease after 3 months (111). Although preliminary, these studies underscore the importance of blood and urinary ketones to monitor the metabolic effects of KDs. However, variability in total caloric content, fats:proteins:carbohydrates ratio, diet duration, and combination with other therapies across several studies makes it difficult to derive conclusions about individual KDs. Prospective trials are currently testing the efficacy of different KDs in combination with chemotherapy and radiotherapy in patients with GBM.

Looking for the Most Effective KD

Two crucial variables potentially affecting the impact of KDs on tumor metabolism are total calorie content and percent macronutrient composition. One study tested the role of moderate, chronic caloric restriction (40% daily

reduction of total calories for 13 days) on blood glucose and IGF1 in mice (112). Across different dietary regimens tested, the calorie-restricted KD with a fats:carbohydrates:proteins ratio of 60:30:10 reduced blood glucose and IGF1 comparably with fasting, and also protected mice from chemotherapy-induced side effects. Although interesting, these results were obtained in animal models and should be confirmed in humans. Moreover, even if two dietary regimens produce similar systemic metabolic changes, their anticancer effects may significantly depend on kinetics of metabolite modifications. For example, fast reduction of glucose, IGF1, and insulinemia as obtained with fasting may be essential to induce metabolic crisis in rapidly proliferating cancers; in contrast, slow reduction of the same metabolites may allow tumors to more easily adapt their metabolism and escape the insult. Because the goal of dietary interventions is to achieve prolonged tumor remissions and major survival effects against a variety of tumors, it is important to (i) test specific dietary interventions in many different types of cancer models *in vivo* and *in vitro*; (ii) combine the diet with different types of standard treatments matching the cancer type (e.g., chemotherapy or TKIs); (iii) generate an intervention which is feasible, safe, and can be easily prescribed.

Fasting-Mimicking Diet

Attempting to design a tolerable diet that modifies systemic metabolism comparably with STF (therefore called "fasting-mimicking diet," or FMD), a standardized, plant-based, low-calorie (500–1,000 Kcal/day), low-protein KD containing a fats:carbohydrates:proteins ratio of around 50:40:10 has been recently developed (113). When administered to healthy human volunteers for 5 consecutive days every month, the FMD reversibly reduced body weight by 15%, and blood glucose and IGF1 by around 11% and 24%, respectively, while increasing ketone bodies and IGFBP1 by around 3- and 1.5-fold. These changes were measured at least 5 days after the subjects had returned to their normal diet, suggesting the effects of the FMD are durable. The FMD is a safer and more acceptable alternative to STF and produces metabolic and antitumor effects comparable to complete fasting, while potentially enhancing effective antitumor immunity (113, 114). As for STF, the impact of the FMD on circulating AAs is still unclear; however, because AAs are crucial for tumor growth, future studies on FMD should prioritize this aspect.

Prospective clinical trials are testing the FMD in combination with chemotherapy in patients with breast cancer.

Protein- and AA-Restricted Diets

Protein-restricted diets could potentially inhibit tumor growth by reducing AA supply to tumor cells and consequently affecting protein synthesis, AA-mediated mTOR activation, and other metabolic processes. Preclinical experiments have shown that protein restriction inhibits *in vivo* tumor growth of melanoma but not breast cancer models, thus indicating its efficacy could be tumor dependent (77). Three other major concerns can be raised against protein restriction in advanced tumors. First, clinical data of safety and efficacy are lacking. Second, nonessential AAs can be *de novo* synthesized by tumor and normal cells from precursors circulating in the blood, thus making general protein restriction unnecessary.

Finally, prolonged protein deprivation can stimulate tumor-induced degradation of muscle cell proteins, thus precipitating sarcopenia on the one side, and overfeeding the tumor with muscle cell–derived AAs on the other side.

Dietary restriction of single AAs could be more safely used to exploit specific tumor auxotrophies. Based on preclinical data, methionine-restricted diets have been tested in populations of patients with advanced cancers and have shown a good tolerability profile (115–117). Overall, circulating blood methionine is reduced by about 60% upon methionine restriction, and some preliminary evidence of antineoplastic activity has also been reported. However, methionine represents an exception to other AAs, whose dietary deprivation is not consistently associated with fast and predictable reduction in blood concentration, and has also raised safety concerns (118). Moreover, normal cells in the tumor microenvironment, including fibroblasts, endothelial cells, and immune cells, can supply tumor cells with AAs deriving from autophagic degradation of their proteins, thus potentially limiting the impact of dietary restriction (119, 120).

Gut Microbiota and Diet

The gut microbiota is being increasingly recognized as a key regulator of the antitumor effect of cytotoxic chemotherapy and novel immune-directed therapies (121). Possible mechanisms to explain this interaction include bacteria-mediated regulation of immune cell activity and metabolic modulation. Indeed, gut-resident bacteria metabolize macromolecules introduced with diet, such as complex carbohydrates and proteins, producing and releasing short-chain fatty acids and AAs into the bloodstream. *In vivo* experiments have shown that mice with intact gut microflora have lower levels of blood tryptophan and N-acetyltryptophan, but higher indoxyl sulfate and indole-3-propionic acid compared with bacteria-free animals (122). Another interesting study demonstrated that caloric restriction can induce microbiota changes that are associated with longer lifespan in mice (123). Future studies should focus on the effect of fasting/FMD on quantitative and qualitative composition of the gut microbiota, because bacterial metabolism could contribute to the metabolic changes occurring in fasting subjects. Moreover, the impact of antibiotics, which are frequently used by patients with cancer to treat tumor-associated or chemotherapy-induced infections, should be further explored, and strategies to repopulate or reshape the gut microbiota, including probiotic supplementation or specific dietary interventions, should be investigated.

PHARMACOLOGIC INTERVENTIONS

Widespread application of dietary interventions may be hindered by serious limitations. First, some subjects may not accept drastic dietary changes, especially fasting. Second, severely calorie-restricted regimens may damage patients with malnourishment or cachexia, a tumor- or chemotherapy-induced deadly syndrome that causes up to 20% of all cancer deaths. Cachexia is characterized by systemic inflammation, damage to normal tissues, and weight loss, and is incompatible with significant caloric restriction, especially in its advanced stages (124). Third, diets can be difficult to standardize, and variability in diet composition or patient

compliance can cause reproducibility problems in clinical trials (107). Finally, some tumors may be specifically addicted to one metabolic pathway, and the pleiotropic effects induced by specific diets may not be strong or selective enough.

Metabolism-targeting pharmacologic therapies are a valuable alternative to dietary approaches and aim to selectively target one specific metabolic pathway. Potential advantages of pharmacologic approaches include the fact that they do not impose significant lifestyle changes to patients and should not induce weight loss. Moreover, their dosages can be precisely titrated and their pharmacokinetic/pharmacodynamic effects reproducibly and precisely determined. The list of chemical compounds targeting tumor metabolism is long and in continuous expansion. In this section, we focus on those molecules that appear as the most promising based on their biochemical mechanisms and preclinical/clinical data of tolerability and efficacy, so as to justify ongoing clinical trials, as summarized in Table 2.

Metformin

Metformin, the reference drug for type II diabetes treatment, inhibits intestinal glucose uptake and liver neoglucogenesis, and also sensitizes peripheral tissues (mainly muscle and fat tissues) to insulin activity (125). In this way, metformin reduces blood glycemia and insulinemia in hyperglycemic/diabetic patients, but not in euglycemic ones. Of note, metformin use in diabetics has been consistently associated with reduced cancer risk (including breast, colon, pancreatic, and liver malignancies) and better cancer prognosis compared with other antidiabetic treatments (126-128).

Potential anticancer mechanisms of metformin include:

1. *Systemic metabolic activities.* Metformin significantly reduces blood glucose, insulinemia, cholesterol, and triglycerides in hyperglycemic/diabetic subjects and in patients experiencing glucocorticoid-induced hyperglycemia (129). In one study on patients with endometrial cancer, it also increased plasmatic ketone body concentration by about 5-fold (130) and has been proposed to reduce IGF1 levels (131). Metformin can therefore potentially target different metabolic pathways that are associated with cancer progression and proliferation, mimicking fasting/FMD; however, different from fasting, most of metformin-induced effects are likely restricted to hyperglycemic/diabetic patients.
2. *Cell autonomous effects.* Metformin has *in vitro* anticancer activity that is likely mediated through inhibition of mitochondrial complex I, reduced NADH oxidation, and increased AMP/ATP ratio. The consequent activation of AMP kinase (AMPK) by its upstream LKB1 kinase results in: (i) inhibition of mTOR signaling and protein translation; (ii) inhibition of ACC and FA synthesis, and upregulation of FA β-oxidation; (iii) inhibition of HMGCR and cholesterol synthesis (125). Metformin-induced shift from anabolic to catabolic processes, together with the energetic stress caused by reduced ATP levels, inhibits proliferation, and, in case of inability to do so, triggers apoptosis in cancer cell lines (132).

To date, only preliminary prospective data support metformin antitumor efficacy in human cancers (133-135). Although several prospective trials are now testing metformin

in advanced tumors, lack of patient selection in most trials may limit their reliability. Disappointingly, three recent studies on patients with advanced pancreatic adenocarcinoma failed to demonstrate improved outcome with metformin in combination with different first- or second-line chemotherapeutic treatments (136-138). In future studies, it will be crucial to select patients who are more likely to benefit from metformin on the basis of systemic metabolic state (e.g., basal glycemia, ketone bodies, insulin, IGF1, and IGFBP levels), tumor genomic/metabolic signature (e.g., activation of the MAPK or IGF1R/PI3K/AKT/mTOR pathways, LKB1 activation state, glycolytic enzyme expression, addiction to specific AAs), and *in vivo* glucose avidity as measured through ^{18}FDG-PET (139). It will also be important to design combinatorial therapies to exploit the potential synergism between metformin and other interventions, such as chemotherapeutic or molecularly targeted drugs (Fig. 1). Finally, the recently described effect of metformin on gut microbiota composition deserves further investigation in light of the potentially significant contribution of intestinal bacteria to systemic metabolism and efficacy of anticancer therapies (140).

Aspirin

Aspirin, the most frequently used nonsteroidal anti-inflammatory drug, has shown interesting metabolic and antitumor properties. In one study on diabetic volunteers, high-dose (6.7 g/day) aspirin taken for 2 weeks significantly reduced fasting glycemia, C reactive protein, total cholesterol, triglycerides, and FAs, with the most common side effects consisting of reversible hearing loss and tinnitus (141). Moreover, low-dose (75-160 mg) daily aspirin has been convincingly associated with reduced cancer incidence and mortality in prospective trials (142, 143). Finally, retrospective studies correlated aspirin use with reduced recurrence of *PIK3CA*-mutated, surgically resected colorectal cancers (144).

Putative antitumor mechanisms of aspirin include:

1. *Systemic activities.* Low-dose aspirin irreversibly inhibits cyclooxygenase 1 (COX1) enzyme and thromboxane A production in platelets, thus preventing platelet clot formation and, possibly, tumor cell migration and metastasis (145). At higher dosages, aspirin reduces systemic and local inflammation and potentially affects systemic carbohydrate and lipid metabolism, especially in diabetic subjects.
2. *Cell autonomous effects.* Sodium salicylate, the main aspirin metabolite in human blood, activates AMPK and inhibits mTOR and FA synthesis in cancer cell lines (146). Another proposed anticancer mechanism of aspirin is inhibition of COX2, which can drive carcinogenesis and is often over-expressed in human cancers, including colorectal cancer (147). Of note, *in vitro* antitumor effects of salicylates are achieved with drug concentrations obtainable after administration of high-dose aspirin.

Although it is highly unlikely that high-dose aspirin can be safely administered to patients with cancer for a prolonged time, brief exposures may be tolerated and possibly capable of strong synergistic anticancer effects with chemotherapy, metformin, or specific dietary interventions. The role of low-dose aspirin in established cancers is more uncertain, and ongoing

Table 2. Main metabolic effects and clinical characteristics of metabolism-targeting drugs under clinical investigation[a]

Drug	Metabolic effects	Potential clinical effects	Risks and side effects	Ongoing trials (tumor types)
Metformin	– Reduced glycemia in hyperglycemics – Reduced insulinemia in hyperglycemics – Reduced FA and cholesterol synthesis in liver and tumor cells – Inhibition of mTOR and protein synthesis	– Synergistic with CT, RT, or dietary interventions	– Lactic acidosis if poor liver, kidney, lung, and heart function – Diarrhea	Lung: NCT02019979, NCT02285855, NCT02115464; Prostate: NCT02640534; Breast: NCT01310231; Endometrial: NCT02755844, NCT01797523; Ovarian: NCT02312661, NCT02122185; WDNETs: NCT02294006, NCT02823691
Aspirin	– Reduced glycemia in hyperglycemics – Reduced FA and cholesterol synthesis in liver and tumor cells – Inhibition of mTOR and protein synthesis	– Tumor growth inhibition – Reduced systemic and peritumoral inflammation – Synergistic with CT – Reduced metastasis	– Gastrointestinal bleeding – Liver and kidney damage	Prostate: NCT02420652; Colorectal: NCT02607072, NCT00565708; Breast: NCT02602938, NCT02804815; Lung: NCT01707823; Esophageal: NCT02326779
DCA	– Inhibition of aerobic glycolysis (PDK inhibition)	– Synergistic with RT or CT – Toxic to tumors overexpressing PDK – Synergistic with metformin	– Peripheral neuropathy	H&N: NCT01386632
Gossypol	– Inhibition of aerobic glycolysis (LDHA inhibition)	– Synergistic with metformin	– Fatigue – Anemia – Dyspnea	NSCLC: NCT01977209; B-CLL: NCT01003769
AZD3965	– Inhibition of aerobic glycolysis (MCT1 inhibition)	– Toxic to highly glycolytic tumors	– Unknown	Several advanced tumors: NCT01791595
CB-839	– Inhibition of glutamine metabolism	– Synergistic with CT or inhibitors of aerobic glycolysis	– Unknown	Breast, lung, renal: NCT02771626, NCT02071862; Leukemia: NCT02071927
ADI-PEG	– Degradation of circulating arginine	– Killing of ASS1-defective tumors	– Leukopenia – Fatigue	Liver, lung, uveal melanoma, glioma, and mesothelioma: NCT02029690; Prostate and NSCLC: NCT01497925; Gastrointestinal: NCT02102022; Mesothelioma: NCT02709512
EGCG/green tea extracts	– Inhibition of FASN and de novo synthesis of FAs	– Inhibition of tumor cell proliferation – Synergistic with CT	– Weight loss – Loss of fatty tissue	Breast: NCT00949923; Urothelial: NCT01993966; SCLC: NCT01317953
Statins	– Inhibition of HMG-CoA reductase and de novo synthesis of cholesterol and isoprenoids	– Inhibition of tumor cell proliferation – Synergistic with CT and inhibition of FA neosynthesis	– Myotoxicity – Liver toxicity	Rectal: NCT02161822; NCT02569645; Prostate: NCT01992042; Breast: NCT02483871, NCT02416427; Bladder: NCT02360618
Rapalogs	– Inhibition of protein synthesis – Activation of autophagy	– Inhibition of tumor cell proliferation – Synergistic with metformin or glycolysis inhibitors	– Mucositis – Fatigue	Breast, renal, and pancreatic: NCT02077933; Endometrial and ovarian: NCT02188550; WDNETs: NCT01648465, NCT02294006

Abbreviations: WDNET, well-differentiated neuroendocrine tumor; H&N, head and neck; NSCLC, non-small cell lung cancer; B-CLL, B-cell chronic lymphocytic leukemia; SCLC, small cell lung cancer; CT, chemotherapy; RT, radiotherapy.

[a]Only the most representative ongoing studies are reported, and for each of them the ClinicalTrials.gov identifier is indicated.

prospective studies are investigating it as an adjuvant treatment after radical surgery.

Targeting Aerobic Glycolysis

The most direct way to target exaggerated aerobic glycolysis in tumors is to reduce glucose availability to cancer cells, which can be achieved through either dietary or pharmacologic (e.g., metformin) interventions. However, although diabetic subjects could reasonably obtain a therapeutically relevant reduction of glycemia, euglycemic subjects would hardly benefit from such interventions, because lowering glycemia below 60 to 70 mg/dL for a prolonged period could irreversibly damage normal tissues, including the heart and the brain. In euglycemic cancer patients, pharmacologic targeting of specific glycolytic enzymes could be the best tolerated and most effective option. Several direct or indirect glycolysis inhibitors are under clinical investigation.

Dichloroacetate

The enzyme pyruvate dehydrogenase kinase (PDK) is overexpressed in several tumors, where it inhibits pyruvate dehydrogenase and diverts pyruvate to lactate, thus contributing to their glycolytic phenotype (148). By inhibiting PDK, dichloroacetate (DCA) stimulates the conversion of pyruvate to acetyl-CoA and mitochondrial oxidation of acetyl groups (Fig. 1; ref. 149). Moreover, it induces mitochondrial depolarization and increases production of reactive oxygen species (150). Interestingly, DCA inhibits *in vitro* and *in vivo* (mouse models) tumor growth and could synergize with metformin to cotarget aerobic glycolysis and mitochondrial oxidative phosphorylation (151, 152). Preliminary clinical studies reported on the antitumor efficacy of DCA in human cancers, with some prolonged tumor remissions in highly pretreated patients with GBM (153). Of note, DCA has been used for decades in oral formulation for treating children with inborn mitochondrial defects and is considered safe at standard dosage (154). Prospective trials are currently testing the efficacy of this promising drug in several tumors.

LDHA Inhibitors

LDHA inhibition prevents regeneration of NAD^+, which is crucially required for glycolysis progression (Fig. 1; ref. 16). Clinical trials investigating the LDHA inhibitor gossypol in different solid cancers are ongoing, but serious concerns exist about its poor tolerability and antitumor *in vivo* efficacy (155). Another way to target lactate metabolism is to inhibit its export from cancer cells into the extracellular environment. Indeed, accumulating intracellular lactate forces the reversible reaction catalyzed by LDHA in the direction of pyruvate production, again preventing NAD^+ regeneration. AZD3965 is an inhibitor of the lactate transporter MCT1, which is expressed at high levels on the cell membrane of several tumors, including breast cancer, colorectal cancer, and gliomas; moreover, high MCT1 levels predict poor patient outcomes (156, 157). Clinical studies are testing the tolerability and antitumor effectiveness of AZD3965 in solid tumors.

Targeting Glutamine Metabolism

Conversion of glutamine to α-KG is a two-step process involving glutamate as an intermediate metabolite (Fig. 1).

Inhibitors of GLS, including BPTES and CB-839; GDH, such as GPNA or EGCG; or TAs, such as aminooxyacetate (AOA), could all target the first steps of intracellular glutamine metabolism (32, 158). In a recent study, CB-839 reduced production of glutamine-derived glutamate, glutathione, and TCA intermediates and displayed significant antitumor activity against TNBC cell lines (159). CB-839 is under clinical investigation in several solid and hematologic malignancies. Another recent paper reported GPT2 transaminase overexpression in glutamine-addicted breast cancer cell lines (160). In this study, the general TA inhibitor AOA reduced tumor cell viability, which was rescued by adding aspartate to the growth medium; this confirms the contribution of glutamine to neosynthesis of other AAs (10). Interestingly, AOA has been clinically tested to treat tinnitus, and its toxicity profile is well known (161). Identifying glutamine-dependent tumors on the basis of biological/immunohistochemistry characteristics (such as MYC overexpression) or functional imaging (such as *in vivo* uptake of glutamine analogs) will prove crucial to select patients who are more likely to respond to inhibitors of glutamine metabolism (162).

Methioninase

Although methionine-restricted diets reduce plasmatic methionine concentration by more than 50% after only 1 day (117), longer restriction is not associated with further decrease, likely because muscle protein degradation can compensate for methionine reduction and consequently limit the effectiveness of dietary deprivation (124). Pharmacologic approaches to optimize methionine depletion include the bacterial methionine-degrading enzyme methioninase, which inhibits growth of mouse models of colon, lung, and brain tumors, especially in combination with cytotoxic treatments (163–165). Of note, methioninase reduces blood methionine independently from patient diet, which can be difficult to control. Moreover, methioninase could counteract homeostatic, rebound increases of methionine absorption or systemic production. Finally, methioninase would deplete not only systemic but also intratumoral methionine that could be produced by normal cells in the tumor microenvironment or by subclones of adapted cancer cells. Low half-life and high immunogenicity of methioninase currently limit its long-lasting efficacy (166), but investigating alternative strategies to optimize methionine depletion in advanced human cancers should be encouraged.

ADI-PEG

Depleting circulating arginine is predicted to be lethal for *ASS1*-repressing malignancies. However, limiting dietary arginine intake is unlikely to be effective, because the liver and the kidneys are able to synthesize and release it in the bloodstream. ADI-PEG, a pegylated (polyethylene glycol conjugated) form of the *Mycoplasma*-isolated arginine deiminase (ADI) enzyme that catabolizes arginine to citrulline and ammonia, is a promising arginine-depleting therapy (167). Repeated ADI-PEG administrations are well tolerated and induce rapid and durable plasmatic arginine reduction. Of note, clinically significant disease stabilizations have been observed in advanced melanomas and patients with HCC treated with ADI-PEG monotherapy, and efficacy correlated with prolonged arginine depletion (168). Resistance mechanisms to ADI-PEG include the production of ADI-PEG–inactivating antibodies by

B lymphocytes and derepression of *ASS1* in tumor cells, which become independent from external supply (168–170). Ongoing phase II/III trials are testing ADI-PEG in advanced HCCs, mesotheliomas, and non-small cell lung cancers (NSCLC); based on the experimentally observed synergy between arginine depletion and interference with DNA synthesis/replication in *ASS1*-repressing tumors, some of these studies are testing ADI-PEG in combination with cisplatin and pemetrexed (171).

Targeting FA Metabolism

Because most tumors *de novo* synthesize FAs from intracellular intermediates (Fig. 1), reducing plasmatic triglycerides and cholesterol levels with dietary or pharmacologic interventions is unlikely to effectively target lipid metabolism in tumors. Instead, pharmacologic inhibition of FA synthesis is a potentially more effective strategy. Cerulenin and C75 are well-studied inhibitors of FASN, but they cause severe side effects, including anorexia and loss of adipose mass due to massive lipolysis, which preclude their administration to patients with cancer (172). On the other hand, EGCG, some of its synthetic derivatives, and some flavonoids (such as quercetin and luteonin) seem to be safer FASN inhibitors; developing tolerated and more effective EGCG derivatives is a promising field of current research (173). ACC is another possible pharmacologic target to inhibit FA synthesis (174). Both metformin and salicylates induce phosphorylation and inhibition of ACC in tumor cell lines, and their potential *in vivo* antitumor activity may be in part related to inhibition of FA synthesis (125, 146). Finally, combined inhibition of aerobic glycolysis and glutamine metabolism with dietary or pharmacologic strategies is another way to deplete intracellular FA precursors.

Statins

Based on *in vitro* and preclinical *in vivo* activity, the cholesterol-lowering drugs known as statins have been evaluated as anticancer agents (175). Prospective studies have shown some antitumor activity, especially with lipophilic statins, in patients with advanced malignancies (176, 177). In particular, one study suggested that HMGCR-overexpressing human breast cancers may be especially sensitive to atorvastatin (177). Statins have also shown preliminary synergistic antitumor activity with gefitinib in patients with NSCLC (178). However, clinical benefit in unselected cancer populations has been globally modest so far. Identifying pathologic/biological tumor characteristics that predict benefit from statins (e.g., activation of the RAS/PI3K/AKT pathway or expression levels of HMGCR) and investigating effective concomitant cytotoxic/metabolic therapies will be crucial steps to optimize antitumor therapy with these drugs.

Rapalogs

mTOR inhibition induces antitumor effects by inhibiting protein synthesis and cell proliferation. mTOR inhibitors are globally referred to as "rapalogs," and include the ancestor drug, rapamycin, and its derivatives everolimus and temsirolimus. Everolimus is currently approved for the treatment of advanced renal, well-differentiated neuroendocrine and hormone receptor-positive breast tumors (179). Interestingly, mTOR is also involved in glucose metabolism by stimulating transcription of HIF1α and consequently upregulating

GLUT1. For this reason, a solid rationale exists for contemporaneously inhibiting mTOR and aerobic glycolysis to synergistically kill tumor cells (26). In particular, combining rapalogs with metformin could produce the following synergistic effects: (i) enhanced mTOR inhibition via metformin-induced AMPK activation; (ii) contemporary targeting of protein translation (via mTOR inhibition) and *de novo* synthesis of FAs and cholesterol (via metformin-induced inhibition of ACC and HMGCR); (iii) reduction of rapalog-induced hyperglycemia, which could partially hamper the antitumor activity of mTOR inhibitors. Several phase I/II trials are now testing the combination of rapalogs and metformin in advanced solid tumors.

CONCLUSIONS AND PERSPECTIVES

The rationale behind selectively targeting cancer metabolism while sparing normal tissues relies on the different ways that normal and cancer cells produce and utilize energy and metabolites, and their consequently different responses to starvation conditions.

In this respect, both dietary and pharmacologic interventions are potentially highly effective strategies that could synergize with classic cytotoxic treatments causing oxidative and metabolic stress, or molecularly targeted therapies that inhibit crucial signal transduction pathways. Which, if any, of the two approaches (dietary or pharmacologic) is more effective as an adjuvant to standard treatments is unclear, and will be the focus of intensive future research. Both strategies hold significant advantages and disadvantages (Table 3). Dietary interventions produce pleiotropic effects on different metabolic pathways, with likely broader antineoplastic activity. Moreover, they are cheap and already available. The major limitation of extreme calorie-restricted regimens is their poor acceptability by some subjects or their inapplicability to cachectic patients. Because cachexia usually arises in the latest tumor stages and most patients with metastatic cancer are not cachectic at diagnosis, drastic dietary interventions are more likely to be tolerated and effective when used in the first-line treatment setting.

On the other hand, pharmacologic therapies target tumor-specific metabolic alterations and could be better accepted by patients with cancer, but require synthesis and investigation of highly selective compounds, with significant costs and longer waiting times. Moreover, tumor cell adaptation to inhibition of single metabolic pathways could lead to fast selection of resistant subclones.

Due to recognized intertumor and intratumor heterogeneity, improving metabolic therapies at best will probably require the identification of crucial metabolic alterations in single tumors. For example, *in vivo* glucose avidity as detected by [18]FDG-PET, or overexpression of glycolytic enzymes by immunohistochemistry, may help to identify tumors that are especially sensitive to reduction of blood glycemia and/or metformin treatment. Similarly, glutamine-addicted cancers, as detected by *in vivo* tumor catabolism of [18]F-(2S,4R)4-fluoroglutamine, may respond to inhibitors of glutamine metabolism (162). Finally, low tumor ASS1 levels as detected by immunohistochemistry may predict response to ADI-PEG or other arginine-depleting strategies, and high methionine tumor uptake could be associated with efficacy of methioninase or dietary methionine deprivation (42).

Table 3. Major advantages and disadvantages of dietary and pharmacologic interventions targeting tumor metabolism

Metabolic intervention	Potential advantages	Potential disadvantages
Dietary (fasting, ketogenic diets, FMD)	– Pleiotropic effects on several metabolic pathways – Low costs – Already available – Synergistic with standard treatments	– Poor acceptance by some patients – Poor applicability to cachectic patients – Poor standardization – Absence of dose-response effect
Pharmacologic	– Highly selective to tumor-specific metabolic alterations – Low impact on patient lifestyle – Predictable pharmacokinetic and pharmacodynamic effects – Dose-response effect	– Efficacy restricted to tumors addicted to a specific metabolic pathway – High costs – Long times for drug production, preclinical testing, clinical development – Fast metabolic adaptation and tumor resistance
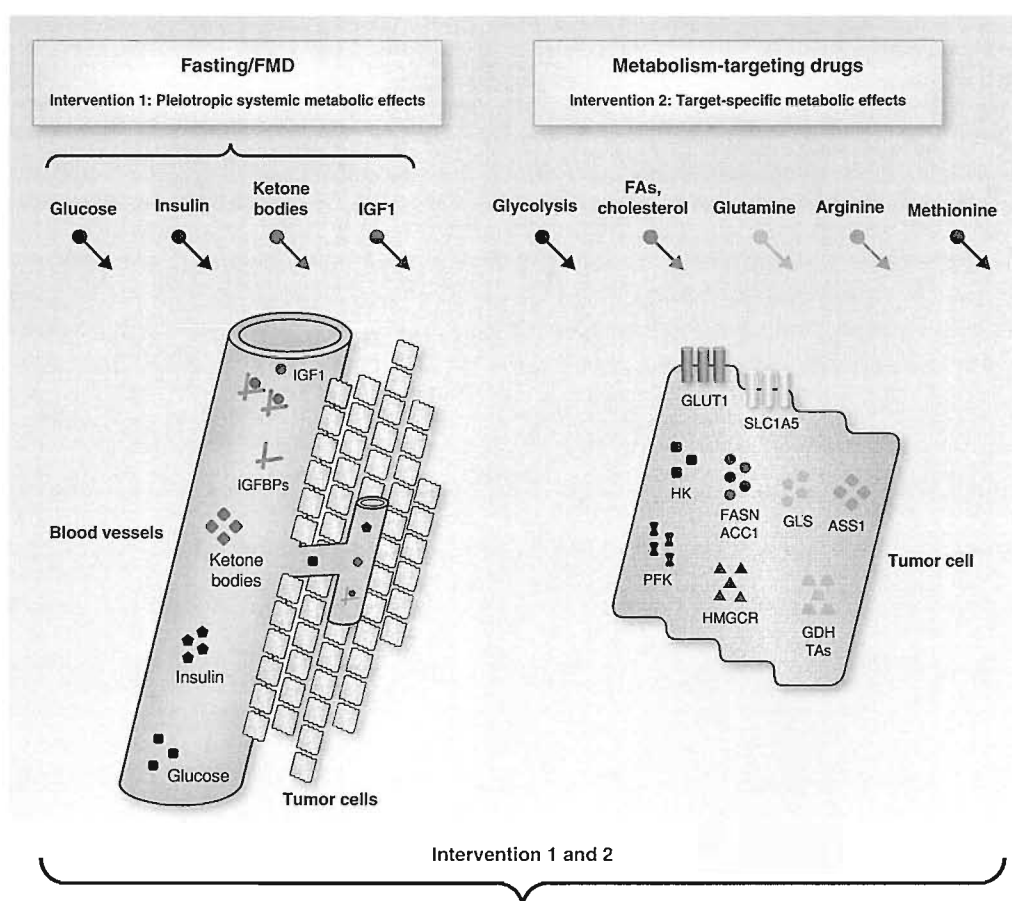
Figure 3. Rationale for combining dietary interventions and drugs targeting specific metabolic pathways in cancers. Fasting and FMD (left) affect systemic metabolism through induction of pleiotropic metabolic effects, including reduction of glycemia, insulin, and IGF1 levels, and increase of ketone bodies and IGFBPs. On the other hand, pharmacologic approaches (right) have the potential to selectively inhibit the specific metabolic pathway(s), such as glycolysis, glutamine, arginine, methionine, FAs, and cholesterol metabolism, to which a single tumor may be addicted. Combining the two strategies could produce synergistic and selective anticancer effects. IGF1, insulin-like growth factor 1; FA, fatty acid; GLUT1, glucose transporter 1; HK, hexokinase; PFK, phosphofructokinase; FASN, fatty acid synthase; ACC1, acetyl-CoA carboxylase; HMGCR, hydroxymethylglutaryl-CoA reductase; GLS, glutaminase; GDH, glutamate dehydrogenase; TA, transaminase; ASS1, argininosuccinate synthase 1.

Combining pleiotropic effects of dietary interventions with pharmacologic targeting of the metabolic pathway(s) to which an individual tumor is addicted may enhance antitumor efficacy of either strategy, while contemporaneously preventing tumor adaptation (Fig. 3).

Disclosure of Potential Conflicts of Interest

Acknowledgments

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