MOLECULAR
METABOLISM

Targeting extracellular nutrient dependencies of cancer cells

Javier Garcia-Bermudez**, Robert T. Williams, Rohiverth Guarecuco, Kıvanç Birsoy*

ABSTRACT

Background: Cancer cells rewire their metabolism to meet the energetic and biosynthetic demands of their high proliferation rates and environment. Metabolic reprogramming of cancer cells may result in strong dependencies on nutrients that could be exploited for therapy. While these dependencies may be in part due to the nutrient environment of tumors, mutations or expression changes in metabolic genes also reprogram metabolic pathways and create addictions to extracellular nutrients.

Scope of review: This review summarizes the major nutrient dependencies of cancer cells focusing on their discovery and potential mechanisms by which metabolites become limiting for tumor growth. We further detail available therapeutic interventions based on these metabolic features and highlight opportunities for restricting nutrient availability as an anti-cancer strategy.

Major conclusions: Strategies to limit nutrients required for tumor growth using dietary interventions or nutrient degrading enzymes have previously been suggested for cancer therapy. The best clinical example of exploiting cancer nutrient dependencies is the treatment of leukemia with L-asparaginase, a first-line chemotherapeutic that depletes serum asparagine. Despite the success of nutrient starvation in blood cancers, it remains unclear whether this approach could be extended to other solid tumors. Systematic studies to identify nutrient dependencies unique to individual tumor types have the potential to discover targets for therapy.

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1. INTRODUCTION

Metabolism comprises the entire set of chemical reactions that occur in a cell. These reactions provide essential building blocks and energy to sustain cellular functions and show remarkable plasticity that allows cells to adapt to stresses in their environments. Metabolic plasticity is particularly important for cancer cells as they experience nutrient and oxygen deprivation in the tumor microenvironment due to a dysfunctional vasculature and high nutrient consumption rates [1]. To survive and proliferate under limited resources, cancer cells rewire their metabolic pathways, use alternative nutrients, and interact with other cell types [2,3].

Mutations or expression changes of metabolic genes reprogram metabolic pathways and impose addictions to non-essential nutrients, which normal cells can synthesize from other sources. Loss of the expression or activity of these metabolic enzymes have marked effects on the levels of metabolite intermediates and precursors, which, in turn, impact other secondary non-metabolic functions [4] (Figure 1). Even in the absence of any metabolic defects, cancer cells often display increased demands for particular nutrients or metabolic by-products generated by other cell types (Figure 1). These unique metabolic features provide the basis for potential anti-cancer therapies. The best clinical example of exploiting cancer nutrient

dependencies is the treatment of leukemia with L-asparaginase, a first-line chemotherapeutic that depletes serum asparagine. Despite the success of nutrient starvation as an anti-cancer approach in this context, it remains unclear whether this approach could be extended to diverse cancer types. In this review, we summarize the dependencies of tumors on major extracellular nutrients, highlighting existing therapies and the potential for depleting nutrients for anti-cancer therapy (Figure 1). Finally, we emphasize the need for identifying such nutrient dependencies to enable the development of future therapies.

2. IMPAIRED SYNTHESIS OF METABOLITES CAN RESULT IN METABOLIC DEPENDENCIES

2.1. Asparagine

The asparagine dependency of blood cancers was fortuitously discovered by John Kidd et al., in 1953. While testing animal sera as a source of complement for the treatment of lymphomas, Kidd et al. found that the serum of guinea pigs, but not that of other animals, caused a strong regression of engrafted mouse lymphomas [5]. Eight years later, JD Broome identified the component of guinea pig serum responsible for tumor regression as asparaginase, an enzyme that effectively depletes serum asparagine [6]. Although earlier studies established the requirement for L-asparagine supplementation for the

Extracellular nutrient dependencies

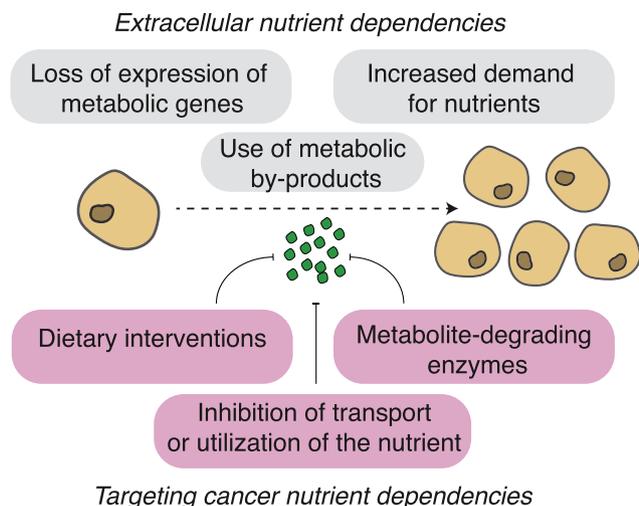*Targeting cancer nutrient dependencies*

Figure 1: Nutrient dependencies of cancer. Oncogenic events can trigger the loss of expression of metabolic enzymes or increase the demand for them. Additionally, tumors may become dependent on metabolic by-products of neighboring or distant cancer and non-malignant cells. These dependencies on extracellular nutrients can be exploited for cancer therapy by nutrient-depleting custom diets, metabolite-degrading enzymes, or blocking their uptake and utilization.

growth of these lymphomas, the discovery of L-asparaginase was the first conclusive demonstration of a tumor metabolic requirement [7] (Figure 2). Initial attempts to exploit this dependency in patients involved the use of guinea pig serum until the isolation of an *Escherichia coli* L-asparaginase, which accelerated the clinical use of the strategy [8,9]. As a monotherapy, L-asparaginase has been shown to cause tumor regression in 20—60% of ALL patients and is a critical component of induction chemotherapy for ALL [10—12].

Unlike leukemias, most human cell types can synthesize asparagine from aspartate through an ATP-dependent reaction catalyzed by asparagine synthetase (ASNS) [13] (Figure 2). Consistent with this, normal cells can survive asparagine depletion by upregulating ASNS transcription and de novo asparagine synthesis. This is mediated by a conserved transcriptional program called the integrated stress response (ISR), which restores homeostasis under a range of physiological stresses. Under nutrient deprivation, the ISR is activated by the accumulation of uncharged tRNAs, which directly activate general control non-derepressible 2 (GCN2), a kinase responsible for the phosphorylation of the eukaryotic initiation factor 2 alpha subunit (eIF2alpha) of the eIF2 complex [14,15]. A major output of ISR is the upregulation of activating transcription factor 4 (ATF4) [16—19]. ATF4 stabilizes and coordinates the upregulation of several nutrient transporters and enzymes, such as ASNS (Figure 2), providing essential nutrients required for survival under nutrient deprivation. The role of asparagine in leukemia cells is likely limited to its proteogenic use in translation as ribosome profiling studies have shown that asparagine limitation halts translation at asparagine residues [20—22]. Indeed, reduction of serum asparagine with L-asparaginase potently restricts global protein synthesis and induces apoptosis in ALL cells [23—25]. Recent work, however, has also highlighted a function of asparagine as an amino acid exchange factor potentially coordinating both protein and nucleotide synthesis [26].

While the precise cause of the asparagine dependencies of leukemias has not been fully elucidated, it has been proposed to involve reduced or complete loss of ASNS expression. Protein levels of ASNS have been shown to strongly correlate with the response to L-asparaginase [27].
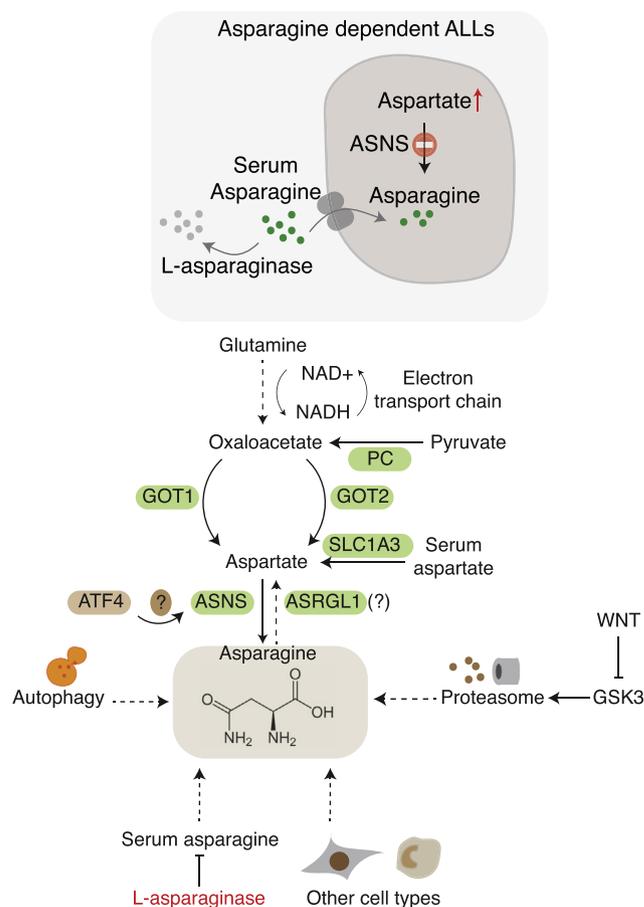

Figure 2: Asparagine availability is dictated by both synthesis and uptake. In acute lymphoblastic leukemia (ALL), cells are unable to synthesize asparagine and rely upon extracellular asparagine for proliferation. L-asparaginase, a bacterial enzyme that depletes serum asparagine, is used in the treatment of ALL to limit extracellular asparagine and inhibit cancer cell proliferation. Asparagine is synthesized from aspartate by asparagine synthetase (ASNS), whose expression is controlled by transcription factor ATF4. Aspartate is derived from anaplerotic substrates such as glutamine or pyruvate through aspartate aminotransferases (GOT1 and GOT2) or the enzyme pyruvate carboxylase (PC), respectively. Exogenous aspartate can also be taken up from serum through aspartate transporters (SLC1A3). Alternatively, asparagine may be recycled through autophagy or proteasomal mechanisms. Finally, asparagine may be imported from the serum or provided by adjacent cell types.

In 1969, Haskell et al. reported a positive correlation between ASNS enzyme activity and L-asparaginase resistance [28]. Likewise, a study of 60 human cancer cell lines from the National Cancer Institute revealed that ASNS cDNA levels negatively correlate with sensitivity to L-asparaginase, a correlation that was even stronger among leukemic cell lines [29]. Furthermore, a competitive growth assay for 554 barcoded cell lines demonstrated that cell lines with high ASNS expression outcompeted those with low expression [30]. In ALLs, the ASNS promoter is regulated through methylation of specific histone marks associated with expression. In cell lines with low baseline ASNS expression, hypermethylation of CpG islands within the promoter of the ASNS gene has been observed [31,32]. Similarly, a recent study found that T-ALLs exhibit lower levels of ASNS due to hypermethylation of the ASNS promoter, which is associated with better outcomes after asparaginase therapy [33]. Interestingly, why ALLs lose or reduce their expression of ASNS remains unclear. It is possible that the expression of ASNS is downregulated at certain stages of hematopoiesis.

Alternatively, loss of ASNS expression may be beneficial in order to maintain higher levels of aspartate (Figure 2), a growth-limiting metabolite for some tumors [34–36]. Taken together, these findings suggest that ASNS is likely the primary cell autonomous determinant of L-asparaginase sensitivity in leukemia. However, conflicting results have also been drawn in correlating ASNS expression to L-asparaginase sensitivity in ALLs [37–40]. For example, a study of clinical ALL samples found no correlation between baseline ASNS expression and response to L-asparaginase in vitro [41]. This discrepancy may be explained in part by alternative cellular sources of asparagine from catabolic protein degradation. Indeed, the proteasomal degradation pathway may provide sufficient asparagine to leukemia cells under asparaginase treatment (Figure 2). Activation of WNT-dependent inhibition of proteasomal degradation improves the therapeutic index of asparaginase [42]. Other cell types in the bone marrow environment may also provide asparagine to leukemia cells under asparaginase treatment (Figure 2). For example, bone marrow-derived mesenchymal cells express ASNS at high levels, and expression has been shown to correlate with both synthesis and secretion of asparagine [43,44]. Furthermore, ALL cells secrete insulin-like growth factor (IGF)-binding protein 7 (IGFBP7) when co-cultured with bone marrow stromal cells to stimulate ASNS expression and asparagine secretion [45]. Adipocytes may also provide anaplerotic substrates to leukemic cells and counteract the effects of L-asparaginase [46]. These studies suggest potential resistance mechanisms for L-asparaginase therapy.

Building upon the success of L-asparaginase for the treatment of blood cancers, there is growing interest in its use for the treatment of solid tumors. Early clinical trials of L-asparaginase across neoplastic diseases found a partial response in melanoma and lymphoma patients [11]. Additionally, hepatocellular carcinomas and gastric and pancreatic cancers with low expression of ASNS exhibit sensitivity to L-asparaginase [30,47,48]. Asparagine availability strongly regulates metastatic potential as L-asparaginase treatment reduces the epithelial-to-mesenchymal transition, invasiveness, and metastatic progression [49]. It should be noted that administration of L-asparaginase may also result in significant depletion of serum glutamine levels at higher doses [50,51]. However, in cells expressing low levels of ASNS, the asparaginase activity, and not the glutaminase activity of L-asparaginase, is sufficient to induce cell death [52]. To overcome this challenge, L-asparaginases have been engineered with negligible off-target glutaminase activity, which may reduce the toxic side effects associated with therapy [53].

2.2. Arginine

Arginine is a nonessential amino acid and a major biosynthetic precursor for a wide range of metabolic reactions. In addition to its proteinogenic role, arginine is required for the synthesis of creatine, polyamines, nitric oxide (NO), agmatine, and urea, with glutamate and proline as dietary precursors. While diet can provide sufficient levels of arginine, many human cells synthesize it from aspartate and citrulline through two major cytosolic urea cycle enzymes, argininosuccinate synthetase (ASS1) and argininosuccinate lyase (ASL) (Figure 3). In the first and rate-limiting reaction [54], ASS1 catalyzes the conversion of amino acids aspartate and citrulline into argininosuccinate prior to its conversion by ASL into fumarate and arginine. Accumulating evidence suggests that some cancer cells, unlike their normal counterparts, are unable to synthesize sufficient arginine and become dependent on extracellular levels to proliferate. This dependency on arginine was first recognized in 1947 [55]. Bach et al. observed a strong inhibition of tumor growth upon injection of purified arginase [56], an enzyme that catalyzes the conversion of arginine into ornithine and urea (Figure 3).

Similar results were obtained later using arginine deiminase (ADI) purified from *Mycoplasma arginini*, a 300-fold more efficient enzyme than arginase at depleting arginine [57]. In hepatocellular carcinoma and melanoma, ADI treatment reduces in vitro and in vivo proliferation [58]. These anti-proliferative effects could be reversed by the addition of supraphysiological concentrations of arginine [59] or overexpression of ASS1 [60]. The recombinant forms of ADI are currently in clinical trials to treat arginine auxotrophic hepatocellular carcinomas, melanomas, acute myeloid leukemias, and colorectal tumors [61–63].

The arginine dependency of many human cancers results from a decrease in ASS1 expression (Figure 3). Reduced or loss of ASS1 expression has been observed in melanoma [64], hepatocellular carcinoma [64], lymphoma [65], and prostate cancer [66], but also often in renal cell carcinoma [67], pancreatic ductal adenocarcinoma [68], ovarian cancer [69,70], lung pleural mesotheliomas [71], or sarcomas and invasive breast carcinomas [72]. While a major mechanism for ASS1 silencing is hypermethylation of its promoter [65,70,71], other mechanisms have been proposed. In fumarate hydratase (FH) deficient tumors, accumulation of fumarate reverts ASL reactions and results in the consumption of cellular arginine, making these cancers arginine auxotrophic [73]. Indeed, treatment of FH null cells with ADI decreases
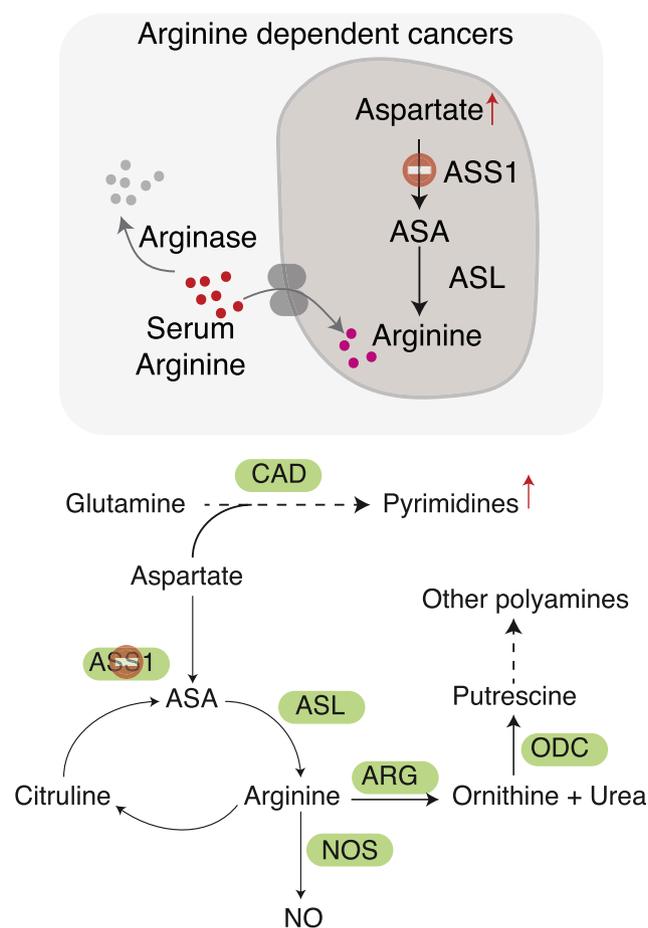

Figure 3: Arginine dependency of ASS1-deficient cancers. Some tumors become dependent on exogenous arginine due to low expression of ASS1, the enzyme catalyzing the first step of the de novo synthesis of arginine from aspartate. As a consequence, depletion of circulating arginine by arginase treatment impairs growth of these tumors. Loss of ASS1 may shunt accumulated aspartate into pyrimidine synthesis and may also decrease the synthesis of arginine metabolism by-products such as nitric oxide (NO) and polyamines.

intracellular arginine levels and inhibits their proliferation. Arginine dependency has recently been associated with the Myc oncogene status of small cell lung carcinomas (SCLC) [74]. Myc-driven mouse and human SCLCs display altered arginine metabolism and are sensitive to ADI treatment. Importantly, upon depletion of arginine, synthesis of polyamines may be the growth-limiting factor for these lung tumors [74]. Arginine depletion also reduces the synthesis of nitric oxide [75], a key promoter of tumor survival and angiogenesis (Figure 3) [76,77]. Finally, prolonged arginine depletion may result in the autophagy-dependent death of ASS1-deficient breast cancers by impairing the mitochondrial integrity and increasing the reactive oxygen species (ROS) [78]. This suggests that arginine depletion may suppress tumor growth by impacting multiple cellular and metabolic processes.

The fact that ASS1 expression is frequently lost in many cancer types suggests that blocking this step in the arginine biosynthetic pathway may be beneficial for tumor growth. The reason for ASS1 downregulation in cancer cells is unknown. ASS1 downregulation may increase the uptake of exogenous arginine, which could be shunted to different metabolic fates such as the production of NO or polyamines [79]. Alternatively, a recent report showed that the accumulation of

aspartate, an ASS1 substrate, and its diversion into nucleotide synthesis promotes ASS1-deficient cancer proliferation (Figure 3) [35,80]. This higher flux into nucleotide synthesis would explain why some ASS1-deficient cancers are resistant to chemotherapy and platinum salts, agents that target DNA repair [70,81].

Without the ability to synthesize asparagine, ASS1 deficient cancers rely on extracellular arginine sources from serum or other cell types (such as macrophages and CAFs) for growth [68,82]. A recent study showed that sustaining circulating arginine levels requires a functional host autophagy in mice. Indeed, defective host autophagy reduces serum arginine levels and suppresses the growth of ASS1-deficient melanomas [83], raising the possibility that autophagy inhibition may also be an effective therapy for arginine auxotrophic cancers.

2.3. Cholesterol
Cholesterol is an essential structural component of cellular membranes. As lipoproteins are major cholesterol carriers in the serum, many human cells rely on the endocytosis-mediated uptake of lipoproteins through low-density lipoprotein receptor (LDLR) to meet their cholesterol demands (Figure 4) [84]. Additionally, cholesterol can be synthesized from
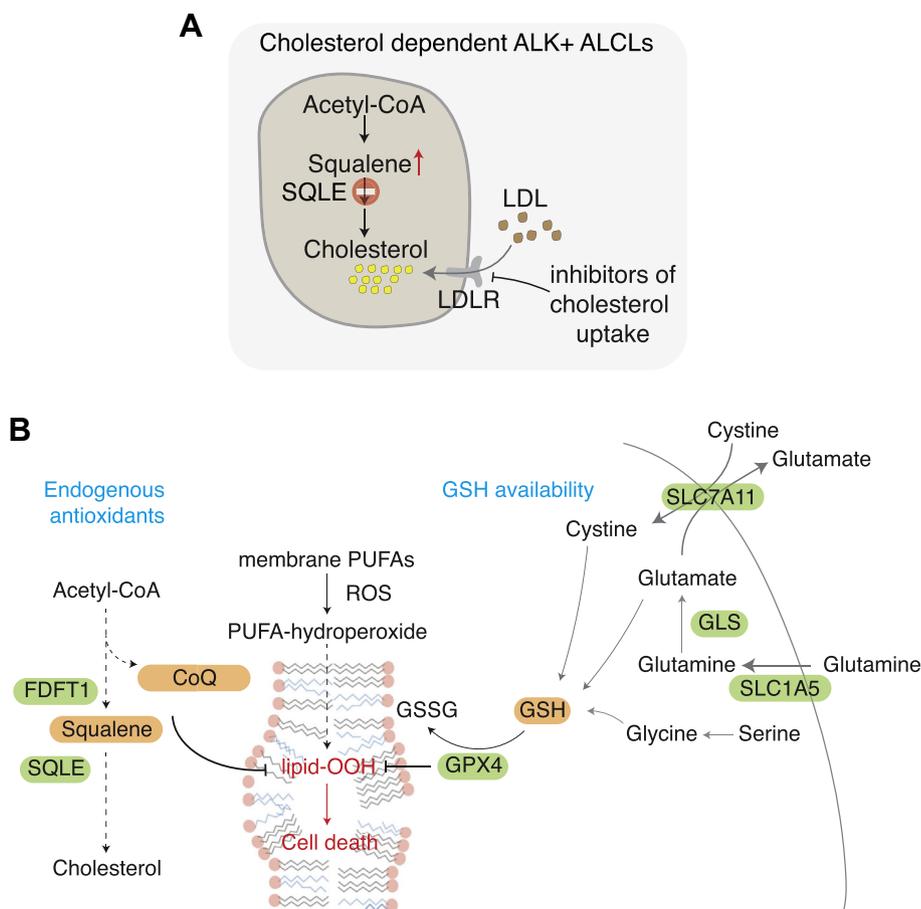

Figure 4: Cholesterol auxotrophic lymphomas accumulate squalene, an antioxidant-like metabolite. A) ALK-positive anaplastic large cell lymphomas (ALK+ ALCLs) are unable to synthesize cholesterol upon the loss of expression of squalene monooxygenase (SQLE), which triggers the accumulation of the cholesterol intermediate squalene. As a consequence, in order to proliferate, these cancers depend on the uptake of cholesterol through the low-density lipoprotein receptor (LDLR). **B)** Mechanisms that prevent cellular lipid peroxidation. Certain mevalonate pathway intermediates, such as squalene or coenzyme Q, can protect cells from cell death triggered by the peroxidation of polyunsaturated fatty acids (PUFAs). Conversely, the functioning of glutathione peroxidase 4 (GPX4), the major enzyme involved in detoxification of lipid peroxides, depends on the availability of reduced glutathione (GSH) levels. GSH is synthesized from three amino acids: glycine, glutamate, and cysteine. Disruption of the uptake or utilization of these immediate precursors decreases GSH synthesis and depletes the antioxidant capacity of cells.

acetyl-CoA in the endoplasmic reticulum through consecutive enzymatic reactions (Figure 4) [85]. Cells maintain cholesterol homeostasis through an integral membrane protein, SREBP cleavage-activating protein (SCAP). SCAP contains a cholesterol-binding pocket and, upon cholesterol shortage, cleaves the sterol regulatory element-binding protein (SREBP) transcription factors, which translocate into the nucleus and promote the transcription of key cholesterol metabolism genes [86]. Because of their high proliferation rates, cancer cells have an increased requirement for cholesterol and many cholesterol genes are downstream effectors of oncogenic signals. Activation of the Pi3K/AKT signaling pathway, for example, induces cholesterol synthesis by activating SREBP and promotes tumor growth and metastasis [87—89]. Similarly, mutant p53 in breast tumors is associated with an increase in cholesterol biosynthesis genes [90].

Paradoxically, cholesterol auxotrophy is also observed in some tumors as a rare phenotypic trait. One of the first examples of cholesterol auxotrophy in cancer was observed in a histiocytic lymphoma cell line, U-937. These cells are cholesterol auxotrophic due to a defect in 3-ketosteroid reductase (HSD17B7) and die in culture unless supplemented with cholesterol [91,92]. Using a DNA barcode-competition assay, our group recently identified similar cholesterol auxotrophic cell lines from several cancer types, including gastric cancer, myeloma, Burkitt's lymphoma, and anaplastic lymphoma kinase (ALK)-positive anaplastic large cell lymphoma (ALK+ ALCL) [93]. Intriguingly, ALK+ ALCL cancer cell lines and primary tumors lose the expression of a rate-limiting cholesterol biosynthesis enzyme, squalene monooxygenase (SQLE) (Figure 4). Consistent with the loss of SQLE expression, these cancers accumulate massive levels of squalene, a cholesterol metabolism intermediate with antioxidant properties [93] (Figure 4). The role of squalene as a lipophilic antioxidant is consistent with previous reports revealing the benefit of endogenous antioxidants for tumor growth and metastasis [94—98]. Similar to squalene or mevalonate intermediates (i.e., coenzyme Q10) [99], there are likely other non-polar endogenous antioxidants that promote primary and metastatic tumor growth.

Most prior attempts to target cholesterol metabolism have focused on the use of statins, a clinical inhibitor of HMG-CoA reductase [100,101]. While statin use is associated with reduced cancer-related mortality for some cancer types [102], it remains unclear whether these effects are cell-autonomous or which tumor types would be most sensitive to such therapy. A recent study identified a strong dependency of gliomas on de novo cholesterol synthesis. Disruption of this process using the menin inhibitor MI-2, which directly inhibits the cholesterol-synthesis enzyme lanosterol synthase (LS), strongly impairs glioma tumor growth and indicates cholesterol synthesis as an attractive target pathway [103]. In other cancer types, such as pancreatic cancers, inhibition of cholesterol uptake through blocking LDLR has also been suggested as an anti-cancer strategy [104,105]. Similarly, in ALK+ ALCLs that are unable to perform de novo cholesterol synthesis, disruption of cholesterol uptake through inhibition of LDLR strongly abrogates their growth in vivo (Figure 4) [93]. This indicates that the cell of origin or mutational landscape can force tumor cells to obtain cholesterol via different metabolic routes.

3. INCREASED DEMAND FOR NUTRIENTS MAY LEAD TO METABOLIC DEPENDENCIES

3.1. Cysteine

Cysteine is a sulfur containing amino acid with diverse roles in protein function and oxidative metabolism. Cysteines in proteins can generate disulfide bonds essential for protein folding. Cysteine availability also impacts iron sulfur cluster containing proteins [106], which are involved in oxidative phosphorylation, heme biosynthesis, and central carbon metabolism. In addition to its proteinogenic role, cysteine contributes to the synthesis of intracellular metabolites such as taurine, coenzyme A, and glutathione (GSH), a tripeptide antioxidant [107]. Among these, glutathione synthesis is the most studied function of cysteine metabolism and its levels strongly depend on cysteine availability (Figure 4).

Most cellular cysteines are acquired from the extracellular environment in the form of cystine, the oxidized dipeptide form of cysteine, by the solute carrier family 7 member 11 (SLC7A11), often referred as system xCT [108]. Alternatively, cysteine can also be synthesized de novo from methionine and serine through the trans-sulfuration pathway [109]. In the de novo synthesis of cysteine, methionine-derived homocysteine is conjugated to serine by the enzyme cystathionine beta-synthase (CBS), generating an intermediate, cystathionine, which in turn is hydrolyzed into cysteine, ammonia, and 2-ketobutyrate in a reaction catalyzed by cystathionine gamma-lyase (CTH). In 1966, Eagle and colleagues observed that some human cells could not grow in cystine-free media even in the presence of L-cystathionine, indicating a block in the trans-sulfuration pathway [110]. Similarly, other groups found that leukemic cells could not grow without cystine in the media, whereas healthy lymphocytes proliferated normally in the absence of environmental cystine [111,112]. Despite their ability to synthesize cysteine, most cells require cystine uptake to proliferate. This dependency is in part due to a decrease in the expression of the trans-sulfuration pathway enzymes in cancer cells [113,114] and to an insufficiency in the de novo synthesis. Blocking cystine uptake increases lipid peroxides and triggers an iron-dependent non-apoptotic type of cell death called ferroptosis by inhibiting a major lipid peroxidase, glutathione peroxidase 4 (GPX4), which requires GSH as a cofactor (Figure 4) [115]. In addition to being a substrate for GSH synthesis, cysteine also prevents ferroptotic cell death in its role as a sulfur donor in the iron-sulfur cluster biosynthesis. In the context of tumors exposed to high-oxygen environments, the cysteine desulfurase NFS1, which uses cysteine as a substrate to donate sulfur for the formation of iron-sulfur clusters, is essential for maintaining cellular health upon lipid peroxidation [116]. Loss of NFS1 results in decreased iron-sulfur cluster synthesis and an increase in free iron, which induces lipid peroxidation and ferroptosis under high oxygen tension [116]. To maintain cellular cysteine levels, several cancers upregulate cysteine transport [117] or, in some cases, decrease its consumption [118].

Targeting cystine uptake has recently been suggested as a strategy for inhibiting the proliferation of glioblastoma [119], pancreas [120], prostate [121], and breast cancer [122]. While targeting xCT has been achieved pharmacologically using drugs such as the anti-inflammatory drug sulfasalazine [123], its efficacy in vivo remains unclear. This has led to the design of a cystathionine gamma-lyase enzyme with a much higher affinity for L-cysteine than for L-cystathionine [124]. Similar to L-asparaginase and arginase enzymes, cyst(e)inase can be delivered in vivo and deplete serum cystine. The potential of this treatment has already been shown in prostate cancer xenografts, with animals receiving this treatment presenting tumors with lower GSH levels, high ROS production, and impaired growth [124]. In addition to its effect on solid cancers, a complementary study showed that the treatment of acute myeloid leukemias with cyst(e)inase almost completely eradicated leukemia stem cells and may be a useful therapeutic strategy [125].

3.2. Glutamine/glutamate

While glutamine is a non-essential amino acid that can be de novo synthesized, most cancer cells depend on it to proliferate in culture [126−128]. This universal dependency is rooted in glutamine's role in central carbon metabolism. Glutamine feeds the TCA cycle and is a key nitrogen donor for the synthesis of purine and pyrimidine nucleotides [129], the cofactor nicotinamide adenine dinucleotide (NAD), and glucosamine-6-phosphate, a precursor for N-linked glycosylation [126]. Moreover, glutamine contributes to the de novo synthesis of other non-essential amino acids such as asparagine, glutamate, proline, aspartate, serine, alanine, and ornithine [128]. Consistent with these essential roles, depletion of glutamine strongly impacts proliferation in vitro. However, in vivo glutamine utilization differs among tumor types, at least at the entry level into the TCA cycle, where glutamine contribution is minimal [130,131], with the notable exception of pancreatic cancers [130]. Indeed, glutaminase inhibitors, which block glutamate synthesis from glutamine, show minimal efficacy in vivo despite their strong anti-proliferative effects in culture. Glutaminase inhibition with a phase 2 clinical drug, CB-839, strongly impairs the in vitro proliferation of PDAC cells but does not impact tumor growth in an autochthonous PDAC model [132]. This raises the possibility that in vivo compensatory mechanisms exist to manage the oxidative stress triggered by impaired glutamine utilization [132].

Glutamine-derived glutamate is one of the three amino acids required for GSH synthesis, but it also facilitates the uptake of cystine, another GSH precursor, from the extracellular environment through its use as an antiporter metabolite by system xCT (SLC7A11) (Figure 4). Glutamine's role in GSH synthesis creates an exploitable dependency when cancer cells are wired to have an increased antioxidant response. For example, loss of Kelch-like ECH-associated protein 1 (Keap1), a negative regulator of nuclear factor erythroid-2-related factor 2 (NRF2; encoded by *NFE2L2*), the master regulator of the cellular antioxidant response [133], increases the dependency of lung cancer cells on glutamine, likely due to an increased demand for GSH synthesis [95]. Treatment of these cancer cells in vitro and in vivo with glutaminase inhibitors strongly inhibits their proliferation [95]. Mechanistically, the inhibition of glutamine metabolism decreases intracellular glutamate levels and impairs cystine uptake, leading to a depletion of cellular GSH pools [134,135]. Similarly, high levels of environmental cystine increase glutamine consumption and glutamate secretion through xCT and sensitize lung cancer cells to glutaminase inhibition [136]. Finally, a study assessing cancer cell dependencies on extracellular glutamine identified a subset of triple-negative breast cancers auxotrophic for glutamine [137]. This dependency was mostly due to the cellular need to generate intracellular glutamate and drive sufficient cystine uptake. Consistently, the inhibition of xCT using sulfasalazine decreased the proliferation of these glutamine auxotrophic cancers [137].

3.3. Aspartate

Aspartate is a precursor for asparagine and arginine in addition to its requirement for purine and pyrimidine synthesis (Figure 5). As most human cells are unable to effectively take up aspartate due to the low expression of aspartate/glutamate transporters [138], they depend on aspartate synthesis from oxaloacetate through mitochondrial aspartate aminotransferase (GOT2) (Figure 5). Aspartate production can be a metabolic limitation for cancer cell proliferation under certain metabolic stresses. As the substrates for aspartate aminotransferases are synthesized through oxidative steps coupled to NAD^+ availability, impaired regeneration of cellular NAD^+ results in a decrease in aspartate synthesis. Indeed, under electron transport chain inhibition, cytoplasmic aspartate aminotransferase (GOT1) sustains cellular aspartate levels through the reductive carboxylation of glutamine (Figure 5) [139]. GOT1, however, is not capable of producing enough aspartate to support proliferation. This is illustrated by the fact that supplementation of exogenous electron acceptors, such as pyruvate [139] or alpha-ketobutyrate [140], the heterologous expression of a NAD^+ recycling enzyme [141], or increasing aspartate uptake [36] rescue cell proliferation defects under electron transport chain inhibition. These findings are also in agreement with the observation that, in order to proliferate in culture, cells lacking functional mitochondria
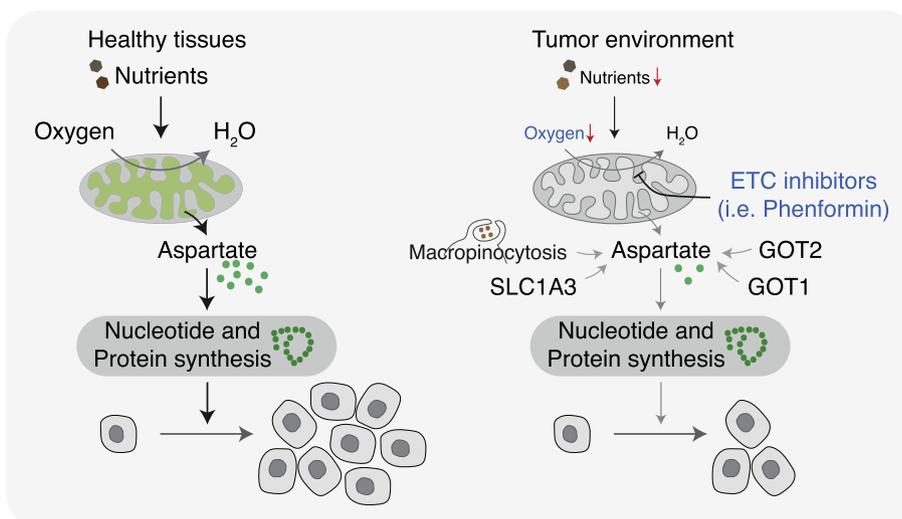

Figure 5: Aspartate is a limiting metabolite in the tumor microenvironment. When oxygen and nutrients are abundant, the mitochondrial electron transport chain (ETC) enables the de novo synthesis of aspartate and its use for the synthesis of new nucleotides and proteins required for cellular proliferation. Cancer cells often experience low nutrient and oxygen environments (right). This stressed microenvironment, in a way similar to cells with ETC defects, decreases aspartate synthesis and imposes the need to obtain aspartate from other sources. In order to meet their aspartate demands, tumors may rely on its de novo synthesis by the aspartate aminotransferases GOT1 and GOT2. Conversely, some tumors are able to take up environmental aspartate through the expression of aspartate transporters such as SLC1A3. KRAS-mutant cancer cells may assimilate aspartate through macropinocytosis, the lysosome-dependent uptake of extracellular proteins.

require the presence of supraphysiological concentrations of pyruvate [142]. Interestingly, cellular aspartate levels and the expression of its transporters can predict the response of different cancer cell lines to electron transport chain (ETC) inhibitors [36]. In complex II deficient kidney cancers, aspartate synthesis is achieved through the carboxylation of pyruvate into oxaloacetate by pyruvate carboxylase (PC) [143] (Figure 5). In addition, under certain conditions, cancer cells downregulate aspartate-consuming pathways to shunt aspartate to nucleotide biosynthesis. For example, cancer cells with low ASS1 expression accumulate aspartate to support proliferation and nucleotide synthesis (Figure 3) [35], demonstrating that different metabolic routes are used to overcome a shortage of aspartate. Under milder ETC dysfunction, aspartate contributes to the formation of oxaloacetate and enables NADH recycling for continuous glycolysis [144]. In malignant cells, aspartate carbons may also be used to replenish the TCA cycle. Indeed, upon glutamine depletion, cancer cells with wild-type p53 can sustain the TCA cycle and proliferation by increasing aspartate availability through the expression of an aspartate transporter [145].

Several studies recently suggested aspartate as a limiting metabolite for the growth of some tumors. Increasing aspartate availability by expressing an aspartate plasma membrane transporter, SLC1A3 [36], or guinea pig asparaginase, an enzyme that metabolizes intracellular asparagine into aspartate, enhances tumor growth [34]. Interestingly, among many metabolites, aspartate abundance correlates best with established transcriptional markers of hypoxia in primary glioblastoma tumors [36], supporting the hypothesis that aspartate synthesis may be impaired in hypoxic tumor environments. Aspartate limitation is likely not common to all cancers, as the growth advantage conferred by increasing aspartate levels is not observed in certain pancreatic cancer cell lines [34,36]. Since pancreatic cancers may use macropinocytosis to scavenge extracellular proteins [146–148], whether these cancers obtain their aspartate through other mechanisms should be determined [149]. Low aspartate levels in tumors likely limit nucleotide synthesis. Indeed, isotope tracing of exogenous aspartate or glutamine reveals a higher incorporation of aspartate to purine and pyrimidines, particularly under low oxygen [36,150]. Interestingly, this essential role in nucleotide synthesis was recently demonstrated by the finding that breast cancer cells lacking functional mitochondria were unable to form tumors due to the impaired functioning of the respiration-linked pyrimidine synthesis enzyme dihydroorotate dehydrogenase (DHODH) [151]. As most cancer types, including hypoxic cancers such as pancreatic ductal adenocarcinoma, generally cannot take up exogenous aspartate from the environment, these cancers may rely on aspartate synthesis or other sources to proliferate. Despite the limiting role of aspartate in tumor growth [34,36], targeting its de novo synthesis in tumors remains untested. Further studies addressing the impact of targeting aspartate metabolism in cancer will clarify the potential of this anti-cancer strategy.

3.4. Serine

Serine is a central biosynthetic metabolite and acts as a precursor for a wide range of biosynthetic reactions. A major role of serine is to provide one-carbon units to folate metabolism by generating 5,10-methylenetetrahydrofolate (CH2-THF), a metabolite with an impact on NADPH production [152–154] that is also essential for nucleotide synthesis. Additionally, serine availability influences epigenetic regulation by coupling the folate cycle with the methionine cycle, allowing the generation of S-adenosyl-methionine (SAM), the methyl donor for DNA and histone methylation [155,156]. Serine also contributes to lipid synthesis as a head group of phospholipids [157] and serves as a

precursor for sphingolipid synthesis [158]. Serine availability is a limiting factor for these processes as its depletion impacts sphingolipid synthesis and impairs the proliferation of yeast [159] and human cancer cells [160]. Serine is used as a precursor for the synthesis of glycine in reactions catalyzed by the mitochondrial enzyme serine hydroxymethyltransferase 2 (SHMT2).

Serine can be either exogenously taken up or synthesized from a glycolytic intermediate (3-phosphoglycerate) in three consecutive reactions that involve phosphoglycerate dehydrogenase (PHDGH) [161], phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH). De novo serine synthesis is upregulated at the gene transcription level or by genomic amplification of the PHDGH locus in some cancer types such as basal-like breast cancers [162] and melanoma [163]. Interestingly, the suppression of PHGDH impacts the proliferation of cancers with elevated PHDGH expression, even in the presence of serine [162]. This raises the possibility that PHDGH expression in these cancers, in addition to driving serine synthesis, may support the TCA cycle anaplerotic generation of alpha-ketoglutarate in transamination reactions catalyzed by PSAT1 [162] and affect central carbon metabolism and the pentose phosphate pathway [164]. Similarly, the overexpression of PHGDH in mouse models of breast cancer and melanoma enhances tumor growth, suggesting that incipient tumors experience periods of serine deprivation in which increased de novo serine synthesis may be beneficial [165].

As PHDGH expression varies greatly across cancer types [162,163], exogenous serine depletion can be used as a therapeutic strategy in cancers with low PHGDH expression [162] or high serine demand. In a p53 null colon cancer model, serine deprivation impaired cell viability due to a decrease in the GSH levels [166]. Activation of p53 in this model triggered the diversion of glucose into serine biosynthesis and the replenishment of GSH pools, increasing survival and proliferation [166], further stressing the potential of lowering serine for the treatment of p53 mutant cancers. A complementary study using serine and a glycine-free (SG-free) diet [167] showed the potential of limiting serine in vivo as an anti-cancer strategy in both a xenograft colorectal cancer model and autochthonous cancer models of adenomatous polyposis coli (Apc)-driven colorectal cancer and c-Myc-driven lymphoma [166,167]. Interestingly, the introduction of an activating mutation of KRAS into these models triggered serine biosynthesis and strongly reverted the anti-tumor effect of the SG-free diet [167]. Deficiency of liver kinase B1 (LKB1), a frequently mutated tumor suppressor gene in pancreatic adenocarcinoma and lung cancer [168], also sensitizes cancer cells to inhibit serine synthesis [156]. Similarly, NRF2, a transcription factor commonly deregulated in lung cancer, activates the expression of PHDGH, PSAT1, and SHMT2 and increases the flux of glucose in serine and glycine biosynthesis [169]. Altogether, these studies suggest that the expression of serine biosynthesis genes and genomic status of the tumors should be considered when targeting serine metabolism.

3.5. Proline

Proline is a key proteinogenic amino acid that is highly abundant in the extracellular matrix component collagen [170]. Proline is synthesized via pyrroline-5-carboxylate reductases and degraded by a mitochondrial enzyme, proline dehydrogenase (PRODH). Both de novo synthesis and degradation of proline involve metabolite intermediate D1-pyrroline-5-carboxylic acid (P5C) and are coupled to cellular energy and redox status, demonstrating the additional functions of this amino acid. During proline degradation, PRODH bound to the mitochondrial inner membrane transfers electrons from proline to a FAD^+ prior to its

transfer to a coenzyme Q pool, enabling ATP production through ETC [171]. In the de novo synthesis of proline, the final step that converts P5C into proline catalyzed by mitochondrial PYCRs (PYCR1 and PYCR2) or cytosolic PYCRL requires reducing the power of NADPH or NADH, depending on the specific isoform [172].

Several cancers depend on the uptake of exogenous proline due to a partial auxotrophy. For example, a human leukemic lymphoblastoid cell line, REH, presents a defect in PYCR activity and completely depends on taking up proline to proliferate [173]. A recent study identified a group of pancreas and lung cancer cell lines as dependent on extracellular proline supplementation [174]. These proline-dependent cancer cell lines exhibited a basal metabolic shunting of glutamine into proline synthesis but failed to trigger the de novo synthesis pathway upon proline depletion. This dependency on exogenous proline was recapitulated in vivo using a proline-free diet [174]. Notably, c-Myc is a known regulator of proline metabolism and plays an important role in activating proline metabolism genes in Burkitt's lymphoma and prostate cancer models [175]. Despite these results, the determinants of proline dependency in vivo have not yet been identified.

Similar to aspartate, proline levels may also limit tumor formation. Ribosome profiling studies to identify tRNA abundance in human clear cell renal cell carcinoma (ccRC) samples revealed enriched signals in proline codons, indicating a proline limitation in vivo [20]. Interestingly, the limitation of proline correlated with an increase in the expression of the de novo proline synthesis enzyme PYCR1, and its knockdown in different ccRC cell lines strongly impaired in vivo tumor formation [20]. These results suggest that proline can limit some primary tumors. Interestingly, matrix metalloproteinases (MMP) can degrade collagen [176], the most abundant protein in the body, and increase proline availability. Because MMP-mediated degradation of ECM is considered pro-tumorigenic in many contexts [177], this raises the possibility that cancer-associated MMPs may provide an important source of proline and thus make tumors less dependent on their synthesis. The energy and redox status can also determine the metabolic route by which cancer cells obtain their proline [178]. For example, an imbalance of the cellular redox state triggered by IDH1 mutation can impact proline metabolism. Indeed, IDH1-mutant glioma cells compensate for this imbalance by increasing proline biosynthesis, in which enhanced PYCR1 activity maintains redox homeostasis [179].

A higher demand of cancer cells for essential amino acids such as methionine [180] or branched-chain amino acids (BCAAs) [181,182] was previously described. This review addresses only the increased dependencies of cancer cells on vitamins.

3.6. Vitamins

Cancer cells may demonstrate increased dependencies on vitamins due to their need for particular vitamin-dependent reactions or because of defects in vitamin transport or activation. Vitamin B9, or folate, is a precursor for tetrahydrofolate, a coenzyme involved in enzymatic transfer of one-carbon groups in various amino acid and nucleic acid synthesis pathways collectively referred to as one-carbon metabolism. As many cancers rely on one-carbon pathways for proliferation [183], targeting one-carbon metabolism pathways with drugs such as methotrexate and 5-fluorouracile (5-FU) has been a successful strategy since the 1940s [184].

Few reports have identified vitamin utilization defects such as impaired transport or inability to synthesize activated derivatives, defects that can be exploited therapeutically. In breast cancer cells, thiamine transporters (SLC19A2 and SLC19A3) have been found to be expressed at lower levels than their normal tissue counterparts. This led to the hypothesis that decreased thiamine transporter expression may make these cancers more dependent on exogenous thiamine, a vulnerability that can be targeted with acute thiamine starvation [185,186]. To circumvent the effects of chronic thiamine starvation, studies utilized a recombinant thiaminase enzyme that digests thiamine and induces an acute thiamine depletion state [187]. One subset of leukemias were more dependent on extracellular thiamine than other tested cell lines [187,188]. In follow-up studies, thiaminase was found to have in vivo efficacy against breast cancer and leukemia subcutaneous xenografts, as well as primary ALL cells injected intravenously [189]. These thiaminase studies provide interesting examples of differential dependencies on extracellular vitamin levels possibly explained by varying degrees of vitamin transport capabilities.

Another vitamin with reported heterogeneity in transporter expression across normal and cancerous tissues is riboflavin. Some riboflavin transporters were observed to be overexpressed in melanoma, breast cancer, and squamous cell carcinoma samples relative to healthy tissues [190]. While these expression patterns may be useful for designing targeted drug-delivery systems, these findings may suggest the existence of cancers with increased riboflavin dependencies. It is also possible for cancers to have vulnerabilities in vitamin utilization downstream of extracellular uptake, such as in the enzymatic conversion of vitamins to activated derivatives. Examples include vitamin B6, an umbrella term for the 6 different vitamers that require different enzymatic steps for interconversion before yielding the activated cofactor form, pyridoxal phosphate (PLP) [191]. Dependencies on exogenous pyridoxal (PL) or PLP forms of vitamin B6 have been observed in some tumors [192]. Overall, with the exception of folate, there are promising but few reports on unique vitamin dependencies in cancer. Studying these dependencies could lead to dietary interventions or new therapeutic targets that could be as impactful as anti-folates have been for the last several decades.

4. CANCER DEPENDENCIES ON METABOLIC BY-PRODUCTS

4.1. Lactate

Cancer cells generally produce high amounts of lactate as a metabolic by-product and secrete it through monocarboxylate transporters (MCTs) [3,193—197]. While lactate production and its reduction from pyruvate are essential for maintaining cellular redox balance, recent studies suggest that extracellular lactate may also be utilized as a carbon source for tumors. For example, some cancer cells in tumors are more oxidative than others and depend on MCT1-mediated lactate uptake to feed mitochondrial oxidative phosphorylation [198]. This dependency is phenocopied in vivo by inhibiting MCT1 activity, which impairs tumor growth and synergizes with radiotherapy [198]. However, the precise contribution of lactate as a fuel for tumors in vivo was an unanswered question until recently. Two studies, one performed in human and mouse lung tumors [199] and the other in mouse cancer models of lung and pancreatic ductal adenocarcinoma [130], showed not only that circulating lactate can be taken up by tumors and used as an anaplerotic substrate for the TCA cycle, but also may be preferred as a substrate over any other carbon source, including glucose [130,199]. Similar to the previous study, MCT1 seems to be the major cell-surface transporter involved in capturing environmental lactate by lung tumors [199]. Altogether, these studies emphasize the need to determine whether lactate is a preferred substrate for some tumors and how secreted lactate from normal tissues and stromal cells may impact tumor growth.

4.2. Acetate

Acetyl-coA is a metabolite involved in diverse cellular functions including central carbon metabolism, lipid and cholesterol synthesis, and post-translational modification of proteins [200]. Acetyl-coA can be produced through glucose or glutamine metabolism via ATP-citrate lyase (ACLY) [201,202]. Alternatively, acetate produced from the diet or the microbiome functions as a precursor of acetyl-coA through reactions catalyzed by acetyl-coenzyme A synthetases (ACSS). While some tumors mostly rely on de novo acetyl-coA synthesis from major carbon sources [201—203], others are avid acetate consumers as observed by positron electron tomography (PET) imaging [204—207] or via nuclear magnetic resonance (NMR) [208]. Studies of hepatocellular carcinoma [209] and glioblastomas [208] show a clear correlation between ACSS2 expression and tumorigenesis in patients and how disruption of ACSS2 expression decreases malignant potential in these cancer models through depleting cellular acetyl-coA levels. Similarly, high ACSS2 expression was observed in patient samples of lung, ovarian, and breast compared to corresponding normal tissue [209], and in the case of triple-negative breast cancer, strongly correlated with poor survival [209]. Acetate is consistently an important carbon source for growth under limiting nutrient and oxygen conditions in several cancer types. Breast cancers show ACSS2 genomic amplification and rely on acetate for most of their fatty acid and phospholipid synthesis during metabolic stresses [210,211]. Complementary studies show that hypoxia not only increases the capture of acetate by cancer cells, but also induces a lipogenic program by increasing histone acetylation [212].

4.3. Ammonia

Ammonia is considered a major metabolic waste product when cancer cells catabolize nitrogenous nutrient sources [213]. Since the de novo synthesis of amino acids and nucleotides requires nitrogen, some cancer cells may rely on reactions that utilize ammonia as a nitrogen source. Two such mechanisms have recently been identified in glioblastoma [214] and breast cancer [215], mediated by glutamine synthetase (GS) and glutamate dehydrogenase (GDH), respectively. In the case of glioblastomas, GS allows the incorporation of ammonia into nitrogen reservoirs by the de novo synthesis of glutamine from glutamate, thus supporting nucleotide synthesis in the context of glutamine starvation [214]. Building upon the observation that ER+ breast cancers express high levels of GDH [216], the second study reported that ammonia is incorporated into glutamate through a GDH-catalyzed reaction of alpha-ketoglutarate and ammonia [215]. In these cancers, very high levels of ammonia accumulate in the tumor interstitial fluid and generate glutamate-derived metabolite products such as proline, aspartate, or glutamine. Knockdown of GDH in an ER+ breast cancer cell line strongly impaired its proliferation in vitro as well as its in vivo growth as tumor xenografts [215], stressing the relevance of this nitrogen-salvaging pathway in this cancer type. Nonetheless, it remains unclear whether this reliance on recycling ammonia as a mechanism to increase nitrogen biomass in the tumor microenvironment occurs in other cancer types or whether the presence of tumor stroma cells may compensate for this dependency by supplying exogenous glutamine to tumor cells. It is also unclear why high levels of ammonia are not toxic in tumors [213] and whether there are adaptative strategies to withstand the anti-proliferative effects of ammonia accumulation.

5. CONCLUSIONS

Cancer cells rewire their metabolic pathways to adapt and survive the demands of high proliferation rates and their environment. Metabolic reprogramming is in part determined by cell-autonomous factors such as the tissue of origin and oncogenic alterations. These metabolic changes often promote tumorigenesis but also provide opportunities for therapy. Indeed, in some cases, cancer cells become dependent on extracellular nutrients to survive and proliferate. These dependencies have been exploited as anticancer therapies for blood cancers, and the discovery of similar nutrient dependencies in other tumor types may result in alternative therapeutic interventions that involve the use of custom diets or metabolite-degrading enzymes.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

None declared.

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