Glutaminolysis: A Hallmark of Cancer Metabolism

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Abstract

Glutamine is the most abundant circulating amino acid in blood and muscle and is critical for many fundamental cell functions in cancer cells, including synthesis of metabolites that maintain mitochondrial metabolism; generation of antioxidants to remove reactive oxygen species; synthesis of nonessential amino acids (NEAAs), purines, pyrimidines, and fatty acids for cellular replication; and activation of cell signaling. In light of the pleiotropic role of glutamine in cancer cells, a comprehensive understanding of glutamine metabolism is essential for the development of metabolic therapeutic strategies for targeting cancer cells. In this article, we review oncogene-, tumor suppressor–, and tumor microenvironment–mediated regulation of glutamine metabolism in cancer cells. We describe the mechanism of glutamine's regulation of tumor proliferation, metastasis, and global methylation. Furthermore, we highlight the therapeutic potential of glutamine metabolism and emphasize that clinical application of in vivo assessment of glutamine metabolism is critical for identifying new ways to treat patients through glutamine-based metabolic therapy.

Contents

1. INTRODUCTION

With significant advances in our mechanistic understanding of cancer initiation, progression, and metastasis in the last decade, the hallmarks of cancer now include maintenance of proliferative signaling, evasion of growth suppressors, resistance to cell death, enabling of replicative immortality, induction of angiogenesis, activation of invasion and metastasis, reprogramming of energy metabolism, and evasion of immune destruction (1). These hallmarks together build up a logical framework to understand the pathology of cancer.

Cancer cells have a sustained chronic proliferative capacity that allows them to grow unhindered, whereas the growth and division of normal tissue are tightly regulated by homeostasis of growth factor signaling. Even though the mechanisms controlling mitogenic signaling in cancer cells vary with distinct oncogenic alterations and differences in the surrounding tumor microenvironment, all cancer cells have a strong propensity to generate macromolecules (e.g., nucleotides, proteins, lipids) from available extracellular nutrients. This ability is enabled via rewired metabolic pathways, which allow significant diversion of nutrient sources to intracellular biomass.

In order to systematically understand the role of metabolic reprogramming in cancer cells on establishing and maintaining a tumorigenic status, Pavlova & Thompson (2) summarized

six emerging hallmarks of cancer metabolism. Among them, dysregulated nutrient uptake and catabolism of glucose and amino acids play a central role in orchestrating intracellular adaptations in metabolic pathways to supply energetically demanding biosynthetic fluxes.

In the 1920s, Warburg (3, 4) discovered that proliferating cancer cells preferentially convert glucose into lactate instead of diverting pyruvate into the tricarboxylic acid cycle (TCA cycle, also known as the Krebs cycle), even in the presence of oxygen. This phenomenon is known as aerobic glycolysis or the Warburg effect, in which only two molecules of adenosine triphosphate (ATP) are produced per molecule of glucose, as compared to the more efficient alternative of glucose oxidation in the TCA cycle that provides 18 times more ATP per glucose molecule. Several theories explaining the mechanism of the Warburg effect have been presented, but convergence to a singular mechanism has been unattainable due to pathological heterogeneity between different tumor types (5). Warburg postulated that mitochondria in cancer cells are defective and that cancer cells therefore reprogram their metabolism to compensate for the damage. In tumors such as hereditary leiomyomatosis, renal cell carcinoma, and gastrointestinal stromal tumor, fumarate hydratase (FH) or succinate dehydrogenase (SDH) is deleted, leading to defective TCA cycle metabolism (6–8). Mitochondrial pyruvate carrier (MPC) 1 and 2, embedded in the mitochondrial inner membrane, are essential for the transport of pyruvate into oxidation through the TCA cycle in yeast, *Drosophila*, and humans (9). MPC mutations also impair pyruvate transport and mitochondrial pyruvate metabolism. However, most tumors have intact mitochondria that are fully functional, indicating that dysfunctional mitochondrial metabolism may not be completely responsible for aerobic glycolysis (10–12). Currently, a widely accepted theory is that aerobic glycolysis can accumulate abundant glycolytic intermediates that are shunted into de novo synthesis of nucleotides, nonessential amino acids, and fatty acids (5). Furthermore, higher glycolytic rates support faster ATP generation compared with glucose oxidation in the TCA cycle (10). In cancer cells in which enzymatic expression of pyruvate kinase isoform 2 (PKM2) is found to be high, the conversion of phosphoenolpyruvate (PEP) into pyruvate is slower than in cells with high PKM1 expression. This leads to the accumulation of pyruvate precursors, which are driven into branch pathways such as the pentose phosphate pathway (PPP), the lipid biosynthesis pathway, or amino acid synthesis.

In order to maintain mitochondrial function under limited pyruvate availability due to aerobic glycolysis, cancer cells replenish TCA cycle metabolites via a process known as anaplerosis. It is critical for cancer cells to maintain their biomass. A close examination of cellular biomass indicates that proteins form the major constituent, accounting for approximately 55% (wt/wt) dry mass. DNA and RNA together constitute 8% of cell mass, whereas other components, including lipids, carbohydrates, and so forth, account for the rest (13). Amino acids are critical for protein assembly and are delivered to the ribosome for protein synthesis by amino acyl–transfer RNA enzymes.

In addition to maintaining biomass precursors, amino acids are involved in multiple metabolic pathways that are essential for cell survival (**Figure 1**). Glutamine is the most abundant circulating amino acid in blood and muscle. Several decades ago, investigators discovered that glutamine consumption rates in HeLa cells are 10 to 100 times greater than those of other amino acids (14). High glutamine consumption has been discovered in many cancers, including pancreatic, ovarian, and breast cancers (15–17). This finding has also been confirmed clinically; plasma glutamine concentration within different tumors is significantly lower than in healthy subjects (18, 19). Therefore, glutamine is a critical amino acid that supports many fundamental cell functions in cancer cells. In this review, we examine several aspects of glutamine metabolism in cancer, including (*a*) oncogenic regulation of glutamine metabolism, (*b*) the contribution of glutamine toward components of cellular biomass, (*c*) sources of glutamine in the tumor microenvironment,

TCA: tricarboxylic acid

ATP: adenosine triphosphate

FH: fumarate hydratase

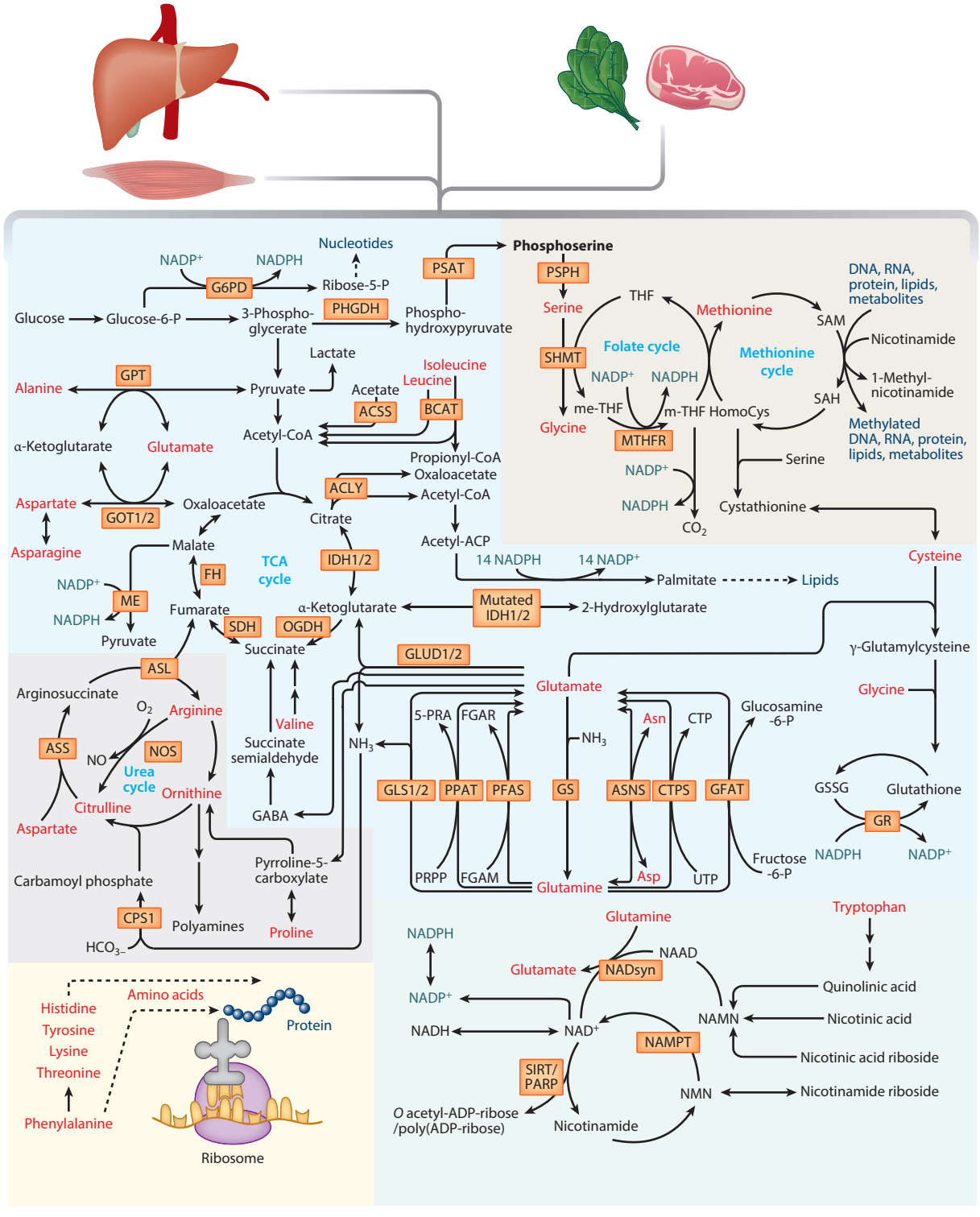
SDH: succinate dehydrogenase

MPC: mitochondrial pyruvate carrier

PKM: pyruvate kinase isoform

PEP: phosphoenolpyruvate

PPP: pentose phosphate pathway


Nucleotides
NADP+
NADPH
G6PD
Ribose-5-P
PSAT
Phosphoserine
Glucose
Glucose-6-P
3-Phospho-glycerate
PHGDH
Phospho-hydroxypyruvate
PSPH
Serine
THF
Methionine
DNA, RNA, protein, lipids, metabolites
SAM
Nicotinamide
Folate cycle
SHMT
Methionine cycle
1-Methyl-nicotinamide
SAH
Lactate
Isoleucine
Leucine
Alanine
GPT
Pyruvate
Acetate
ACSS
BCAT
me-THF
m-THF
HomoCys
Glycine
MTHFR
Methylated DNA, RNA, protein, lipids, metabolites
α-Ketoglutarate
Glutamate
Acetyl-CoA
Propionyl-CoA
Oxaloacetate
Serine
Aspartate
GOT1/2
Oxaloacetate
ACLY
Acetyl-CoA
Cystathionine
Citrate
Asparagine
Malate
Acetyl-ACP
TCA cycle
IDH1/2
14 NADPH
14 NADP+
Cysteine
FH
Palmitate
Lipids
NADP+
ME
NADPH
Fumarate
α-Ketoglutarate
Mutated IDH1/2
2-Hydroxylglutarate
Pyruvate
SDH
OGDH
Succinate
γ-Glutamylcysteine
GLUD1/2
ASL
Glutamate
Glycine
Arginosuccinate
O2
Arginine
5-PRA
FGAR
Asn
CTP
Glucosamine -6-P
Valine
NH3
NH3
ASS
NO
NOS
Succinate semialdehyde
GLS1/2
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PFAS
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ASNS
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GSSG
Glutathione
Urea cycle
Ornithine
Citrulline
GABA
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Glutamine
Asp
UTP
Fructose -6-P
Carbamoyl phosphate
Pyrroline-5-carboxylate
Tryptophan
Glutamine
NADPH
NAAD
Glutamate
NADsyn
Quinolinic acid
NADP+
NAMN
Nicotinic acid
NADH
NAD+
NAMPT
Nicotinic acid riboside
CPS1
Polyamines
Proline
SIRT/ PARP
NMN
Nicotinamide riboside
HCO3−
O acetyl-ADP-ribose /poly(ADP-ribose)
Nicotinamide
Amino acids
Histidine
Tyrosine
Lysine
Threonine
Phenylalanine
Protein
Ribosome

(*d*) the therapeutic potential of targeting glutamine metabolism, and (*e*) clinical applications of in vivo assessment of glutamine metabolism.

2. GLUTAMINE METABOLISM

2.1. Glutamine Anaplerosis Drives the Tricarboxylic Acid Cycle

Many cancer cells undergo metabolic reprogramming that makes them highly glutamine dependent for their survival and proliferation. Deprivation of glutamine in these cells results in growth arrest and cell death owing to their glutamine addiction (16, 20). Glucose-derived pyruvate can be converted into acetyl-CoA through pyruvate dehydrogenase (PDH) in the mitochondria to maintain TCA metabolite pools. However, pyruvate can also directly replenish mitochondrial oxaloacetate (OAA) pools via pyruvate carboxylase (PC) activity. Interestingly, glutamine-independent cancer cells have higher PC expression, which can maintain anaplerosis under glutamine-deprivation conditions. The pyruvate carboxylation reaction is critical for early-stage non-small-cell lung cancer and glutamine-independent tumor cell growth (21, 22).

Glutamine-dependent cell lines consume glutamine as the preferred anaplerotic substrate, as is evident from their OAA pools, 90% of which are derived from glutaminolysis (20). Glutaminolysis is the process by which cells convert glutamine into TCA cycle metabolites through the activity of multiple enzymes. Glutamine is first converted into glutamate via glutaminase (GLS/GLS2). Glutamate is then converted into α-ketoglutarate via two divergent pathways. The first is via the activity of glutamate dehydrogenase (GLUD). The second is via the activity of a group of transaminases, including glutamate–oxaloacetate transaminase (GOT), glutamate–pyruvate transaminase (GPT), and phosphoserine transaminase (PSAT) (**Figure 2**). Flux via GLUD generates the potential autophagy inducer ammonium and the cofactor NADH [reduced form of nicotinamide adenine dinucleotide (NAD)] or NADPH [reduced form of nicotinamide adenine dinucleotide phosphate (NADP)]. By contrast, transaminases promote the generation of other nonessential amino acids (NEAAs), including aspartate, alanine, and phosphoserine. α-Ketoglutarate thus generated can serve as an anaplerotic substrate in the TCA cycle. Citrate can be generated when glutamine-derived OAA condenses with acetyl-CoA. Citrate connects mitochondrial metabolism to de novo lipogenesis via ATP–citrate lyase (ACLY) and fatty acid synthetase (FASN). Furthermore, α-ketoglutarate can also be exported to the cytosol to be carboxylated into citrate through isocitrate dehydrogenase 1 (IDH1) for fatty acid synthesis. TCA cycle metabolites derived from glutamine carbons can also be released into the cytoplasm through mitochondrial shuttles and

PDH: pyruvate dehydrogenase

OAA: oxaloacetate

PC: pyruvate carboxylase

NAD: nicotinamide adenine dinucleotide

NADP: nicotinamide adenine dinucleotide phosphate

NEAAs: nonessential amino acids

GLS: glutaminase

GLUD: glutamate dehydrogenase

GOT: glutamate–oxaloacetate transaminase

GPT: glutamate–pyruvate transaminase

PSAT: phosphoserine transaminase

ACLY: ATP–citrate lyase

FASN: fatty acid synthetase

IDH: isocitrate dehydrogenase

Figure 1

Amino acid metabolic pathways in cancer cells. This detailed schematic depicts the involvement of essential amino acids and nonessential amino acids in protein synthesis, central carbon metabolism, one carbon cycle, the urea cycle, and NAD synthesis. Abbreviations: ACLY, ATP–citrate lyase; ACSS, acetyl-CoA synthetase; ASL, argininosuccinate lyase; ASNS, asparagine synthetase; ASS, argininosuccinate synthase; BCAT, BCAA transaminase; CPSI, carbamoyl phosphate synthetase I; CPTS, carnitine palmitoyltransferase I; FH, fumarate hydratase; G6PD, glucose-6-phosphate dehydrogenase; GFAT, glucosamine-fructose-6-phosphate aminotransferase; GLS, glutaminase; GLUD, glutamate dehydrogenase; GOT, glutamate–oxaloacetate transaminase; GPT, glutamate–pyruvate transaminase; GR, glutathione reductase; GS, glutamine synthetase; IDH, isocitrate dehydrogenase; ME, malic enzyme; MTHFR, methylene tetrahydrofolate reductase; NADsyn, NAD^+ synthetase; NAMPT, nicotinamide phosphoribosyltransferase; NO, nitric oxide; NOS, nitric oxide synthase; OGDH, oxoglutarate dehydrogenase; PFAS, phosphoribosylformylglycinamidine synthase; PARP, poly(ADP ribose) polymerase; PHGDH, phosphoglycerate dehydrogenase; PPAT, phosphoribosyl pyrophosphate aminotransferase; PRPP, 5-phosphoribosyl-α-pyrophosphate; PSAT, phosphoserine aminotransferase; PSPH, phosphoserine phosphatase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SDH, succinate dehydrogenase; SHMT, serine hydroxymethyltransferase; SIRT, sirtuin; TCA, tricarboxylic acid; THF, tetrahydrofolate.

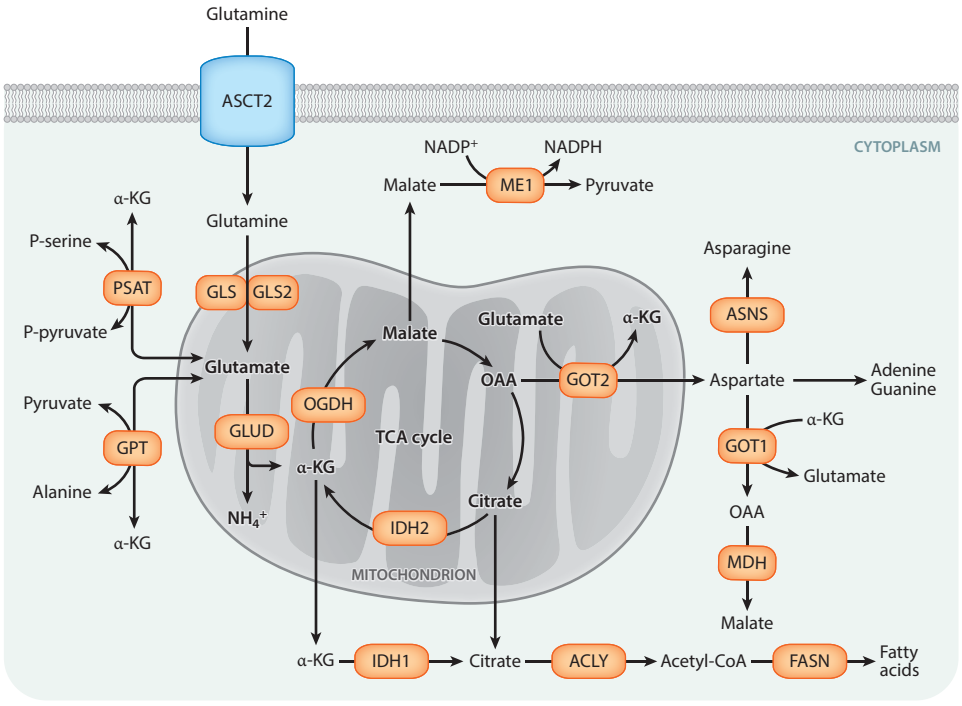


Figure 2

Glutamine anaplerosis into the TCA cycle. Glutamine is taken up via ASCT2 (SLC1A5) and is converted into glutamate. Glutamate is metabolized to α-KG through the action of either GLUD or transaminases. The TCA cycle metabolite malate can be exported out of the cytoplasm to generate NADPH and pyruvate through the activity of the malic enzyme. OAA can be converted back to aspartate, which supports asparagine generation, and nucleotide synthesis. Citrate can be exported out of the mitochondria for de novo fatty acid synthesis. Abbreviations: ACLY, ATP–citrate lyase; ASNS, asparagine synthetase; GLS/GLS2, glutaminase/glutaminase 2; FASN, fatty acid synthetase; GLUD, glutamate dehydrogenase; GOT, glutamate–oxaloacetate transaminase; GPT, glutamate–pyruvate transaminase; IDH, isocitrate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; OAA, oxaloacetate; OGDH, oxoglutarate dehydrogenase; PSAT, phosphoserine transaminase; TCA, tricarboxylic acid; α-KG, α-ketoglutarate.

carriers. For example, the mitochondrial aspartate malate shuttle transfers GOT-derived aspartate out of the mitochondria into the cytoplasm to be converted back into cytoplasmic malate for NADPH generation through malic enzyme 1 (ME1) (**Figure 2**). Aspartate can also be converted into asparagine, incorporated into the urea cycle, or consumed by nucleotide synthesis pathways. Glutamine anaplerosis in the TCA cycle provides critical precursors for NEAAs, nucleotides, and lipids (**Figure 2**).

2.2. Glutamine Metabolism in Organs

Plasma glutamine concentration ranges from 450 to 800 μM in humans (**Table 1**) (18, 23–25). How glutamine is metabolized in the body depends on the specific organ and the cell types within the organ (**Table 1**). In healthy subjects, skeletal muscle cells are the most prolific producers of glutamine. They secrete as much as 59 μmol/min in humans, resulting in higher muscle interstitial concentrations of glutamine than plasma concentrations (26). The regional amino acid

ME: malic enzyme

Table 1 Glutamine metabolism in different organs

Tissue	Type	Extracellular fluid glutamine concentration (μM)	Tissue glutamine concentration (μmol/g tissue)	Glutamine secretion (+)/uptake (−)	Notes
CNS	Astrocyte	472 ± 38 (18)	7.13 ± 0.21 (18)	+	Neurotransmission of glutamine–glutamate cycle (29)
	Neuron			−	
	Neoplasm	377 ± 49.2 (18)	7.00 ± 0.86 (18)	NA	Tumor growth
Adipose	Mature adipocyte	783 ± 4 (23)	NA	+	Differentiation; desensitized to proinflammatory stimuli, lipogenesis (31)
Muscle	Normal	596 ± 60 (24)	3.52 ± 0.17 (32)	+	Chronic uremia enhances glutamine secretion (32)
	Tumor bearing	674 ± 72 (glutamine-enriched diet); 681 ± 71 (glutamine-free diet) (25)	2.31 ± 0.21 (glutamine-enriched diet), 1.44 ± 0.22 (glutamine-free diet) (25)	+	Tumors result in muscle glutamine depletion and weight loss (25)
Liver	Normal	NA	5.7 ± 0.2 (33)	−	Uptake rate: 65.3 ± 8.4 μmol/min (arterial–hepatic venous differences) (34), detoxification and pH homeostasis (35)
	Hepatocellular carcinoma	Decreased plasma glutamine concentration compared with control (19)	0.7 ± 0.1 (rapid growth tumor) (33)	NA	Lower GS than normal, higher GLS than normal (36), tumor growth

Abbreviations: CNS, central nervous system; GLS, glutaminase; GS, glutamine synthetase; NA, not applicable.

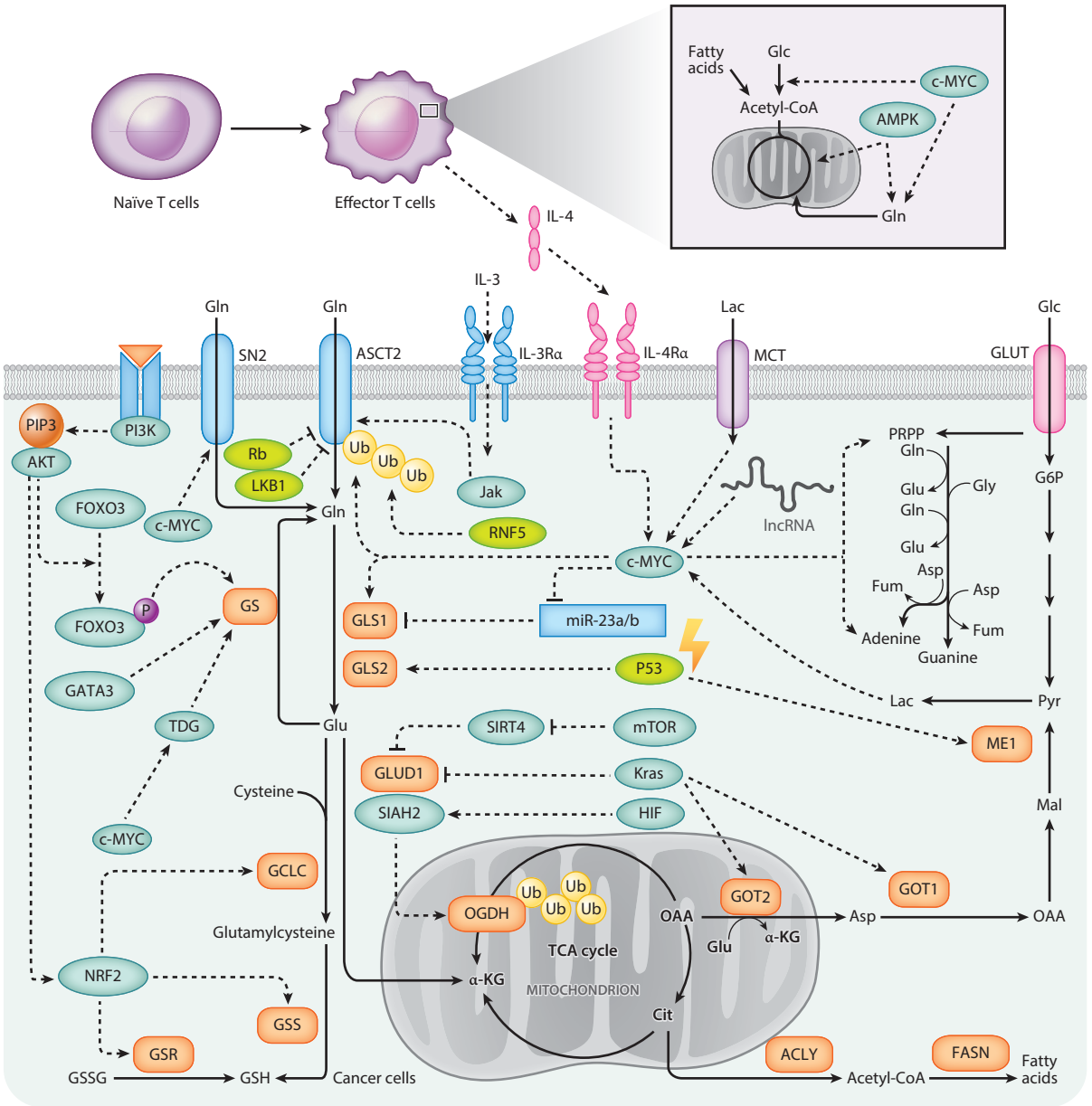
concentration around adipose tissue indicates that adipocytes can also contribute to plasma glutamine with rates of up to 12 μmol/min (27). Furthermore, recent studies have revealed that adipocytes can confer resistance to L-asparaginase in leukemia cells by secreting glutamine (28). Glutamatergic neurons release glutamate into the synaptic cleft, and this glutamate is taken up by astrocytes for glutamine synthesis. Glutamine thus synthesized is shuttled back to the neurons, forming the glutamate/glutamine neuroglial cycle (29). In the normal brain, the glutamine concentration in extracellular fluid can reach 472 ± 38 μM; however, neoplasia results in a reduced extracellular glutamine concentration (**Table 1**) (18). The major organs that consume glutamine are the splanchnic tissues, gut, liver, kidney, and brain, with net uptake of 97, 57, 20, 60, and 13 μmol/min, respectively (30).

2.3. Glutamine Metabolism, Oncogenes, and Tumor Suppressors

Several studies have provided evidence that oncogenic alterations in cancer cells reprogram glutamine metabolism (**Figure 3**) (37, 38). The proto-oncogene *c-MYC* transcriptionally binds to the promoter regions of high-affinity glutamine importers, including *ASCT2* (sodium-dependent neutral amino acid transporter type 2, also known as *SLC1A5*) and *SN2* (isoform of system N,

PI3K: phosphatidylinositol 3-kinase

also known as *SLC38A5*), resulting in higher glutamine uptake (38). Furthermore, overexpression of *c-MYC* promotes the expression of mitochondrial GLS through transcriptional repression of miR-23a and miR-23b (39). *c-MYC* also directly targets genes involved in dNTP (deoxyribonucleotide triphosphate) metabolism to enhance glutaminolysis in order to derive amide nitrogens for nucleotide synthesis (40). Oncogenic *KRAS* elevates the gene expression of enzymes associated with glutamine metabolism in *KRAS*-transformed cells (41). Specifically, *KRAS* downregulates expression of GLUD and upregulates expression of GOT to release aspartate into cytoplasm for NADPH generation via ME1 (37). The phosphatidylinositol 3-kinase/AKT/mechanistic target



of rapamycin (PI3K/AKT/mTOR) pathway is deregulated in several cancers and can influence glutamine metabolism. For example, mTOR complex 1 (mTORC1) activates GDH by transcriptionally repressing SIRT4, a known inhibitor of GDH (42). The PI3K/AKT axis can also activate nuclear factor–like 2 (NRF2), a redox-sensitive transcription factor that regulates redox by multiple mechanisms, including production and regeneration of glutathione (GSH) (43). Glutathione is synthesized from the amino acids glutamate, cysteine, and glycine. Activated NRF2 upregulates the expression of glutamate–cysteine ligase (GCLC) and glutathione synthetase (GSS) to enable GSH production (44). Additionally, NRF2 maintains GSH redox homeostasis by converting glutathione disulfide (GSSG) into reduced GSH, which is catalyzed by glutathione reductase (GR).

Glutamine metabolism can also be regulated by tumor suppressors. The tumor suppressor *p53* activates GLS2 expression and removes intracellular reactive oxygen species (ROS) to protect cells from genomic damage (**Figure 3**) (45, 46). Other tumor suppressors, such as retinoblastoma protein (Rb) and liver kinase B1 (LKB1), alter glutamine uptake by repressing the expression of ASCT2 by E2F transcription factor 3 (E2F3) (**Figure 3**) (47, 48).

In addition to oncogenes and *p53*, glutamine metabolism can be regulated by several other factors. Recent findings have shown that the long noncoding RNA (lncRNA)–prostate cancer gene expression marker 1 (PCGEM1) promotes chromatin recruitment of *c-MYC* to enhance activation of aerobic glycolysis, pentose phosphate shunt, and glutamine metabolism (49). The lncRNA CCAT alleles have distinct affinities for cleavage factor I complex subunits (CFlm25 and CFlm68), resulting in alternative splicing of GLS for GAC (glutaminase C) and KGA (kidney-type GLS) (50). Finally, the ubiquitin ligase ring finger protein (RNF5) regulates the level of glutamine carrier proteins SLC1A5/38A2 by ubiquitination and degradation in response to chemotherapy-induced endoplasmic reticulum (ER) stress (51).

mTORC1: mechanistic target of rapamycin (mTOR) complex 1

NRF2: nuclear factor–like 2

GSH: glutathione

GCLC: glutamate–cysteine ligase

GSS: glutathione synthetase

GSSG: glutathione disulfide

GR: glutathione reductase

ROS: reactive oxygen species

Rb: retinoblastoma protein

LKB1: liver kinase B1

E2F3: E2F transcription factor 3

2.4. Glutamine Metabolism and Tumor Microenvironment

Glutamine metabolism is also profoundly influenced by the extracellular tumor microenvironment (52). Complex metabolic interactions between stromal cells (derived from neighboring tissue or recruited from bone marrow) within the tumor microenvironment and cancer cells can promote tumor growth. Interleukin-4 (IL-4), which is secreted by immune cells, can increase the expression

Figure 3

Oncogenic signaling, tumor suppressor, and tumor microenvironment effects on glutamine metabolism. Expression levels of enzymes involved in the glutaminolysis pathway are regulated by intrinsic genetic mutations and abnormal tumor microenvironments. The tumor suppressors Rb and LKB1 inhibit ASCT2. The oncogene *c-MYC* enhances expression of *ASCT2*, *SN2*, *GLS1*, and genes involved in purine synthesis. *c-MYC* also activates TDG to promote the GS transcriptional level. DNA damage and other stressors stimulate the activation of *p53*, which transcriptionally upregulates GLS2 and ME1 expression. mTOR inhibits SIRT4, which represses GLUD1. KRAS inhibits GLUD1, but enhances GOT1/2 expression. HIF enhances OGDH ubiquitination. The redox-sensitive transcriptional factor NRF2 regulates the expression of GCL, GSS, and GSSR to maintain GSH levels. GS can be transcriptionally upregulated by GATA3, and FOXO3/4, whose phosphorylation is controlled by the PI3K/PKB/AKT signaling cascade. The growth factor IL-4, originating from immune cells, activates c-MYC in cancer cells. The metabolism of immune cells is also controlled by intrinsic c-MYC and AMPK signaling. IL-3 can bind with IL-3Rα and activate Jak to enhance ASCT2 expression. Abbreviations: ACLY, ATP–citrate lyase; AKT (PKB), protein kinase B; AMPK, AMP-activated protein kinase; FASN, fatty acid synthetase; FOXO3, forkhead box protein O3; GATA3, GATA-binding protein 3; GCLC, glutamate–cysteine ligase; GSH, glutathione; GLS/GLS2, glutaminase/glutaminase 2; GLUD 1, glutamate dehydrogenase 1; GOT1/2, glutamate–oxaloacetate transaminase1/2; GS, glutamine synthetase; GSR, glutathione reductase; GSS, glutathione synthetase; HIF, hypoxia-inducible factor; IL, interleukin; Jak, Janus kinase; LKB1, liver kinase B1; ME1, malic enzyme 1; NRF2, nuclear factor (erythroid-derived 2)-like 2; OAA, oxaloacetate; OGDH, oxoglutarate dehydrogenase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PI3K, phosphatidylinositol 3-kinase; Rb, retinoblastoma protein; RNF5, ubiquitin ligase ring finger protein; SIAH2, Siah E3 ubiquitin protein ligase 2; TDG, thymine–DNA glycosylase; Ub, ubiquitin.

level of the glutamine transporter ASCT2 in breast cancer cells (**Figure 3**) (53). Growth factors such as IL-3 that are present in the tissue microenvironment can bind to glucose-sensitive IL-3Rα and promote glutamine uptake by upregulating ASCT2 expression (54). Blocking IL-3 activity using a Jak inhibitor represses glutamine uptake, suggesting that IL-3 acts via the Jak/STAT (signal transducer and activator of transcription) pathway (54). Furthermore, effector cells differentiated from B lymphocytes after activation by antigens require AMP-activated protein kinase (AMPK) and c-MYC to maintain glutaminolysis for consistent secretion of IL-4 (55, 56).

Rapidly growing solid tumors exhibit both spatially and temporally heterogeneous oxygen distribution due to abnormal vasculature. This heterogeneity in oxygen concentration results in hypoxic environments, leading to activation of hypoxia-inducible factor 1 (HIF1) (57). HIF activation reduces pyruvate entry into the mitochondria by decreasing the activity of PDH. Furthermore, activation of HIF1 can promote E3 ubiquitin–protein ligase SIAH2–dependent ubiquitination and subsequent degradation of oxoglutarate dehydrogenase 2 (OGDH2), a key enzyme for α-ketoglutarate conversion into succinate (58). Consequently, under hypoxic conditions, glutamine oxidative metabolism shifts toward reductive metabolism (58). Hypoxic environments also enhance the accumulation of lactate, a by-product of HIF1-induced glycolysis. Lactate in the tumor microenvironment not only results in a "reversed" pH gradient that enables tumor progression but also activates c-MYC, which directly affects glutaminolysis (59).

lncRNA: long noncoding RNA

PCGEM1: prostate cancer gene expression marker 1

RNF5: ubiquitin ligase ring finger protein

ER: endoplasmic reticulum

IL-4: interleukin-4

AMPK: AMP-activated protein kinase

HIF1: hypoxia-inducible factor 1

OGDH: oxoglutarate dehydrogenase

CPSI: carbamoyl phosphate synthetase I

CPSII: carbamoyl phosphate synthetase II

CTPS: cytidine triphosphate synthetase

UTP: uridine triphosphate

PRPP: 5-phosphoribosyl-α-pyrophosphate

PRA: phosphoribosyl-β-amine

PPAT: phosphoribosyl pyrophosphate amidotransferase

FGAR: formylglycinamide ribonucleotide

FGAM: phosphoribosylformylglycinamidine

3. FUNCTIONAL ROLES OF GLUTAMINE

3.1. Glutamine as a Nitrogen Donor for Purines and Pyrimidines

In addition to its role in TCA cycle anaplerosis and protein synthesis, glutamine serves as a critical nitrogen donor. The deamination of glutamine into glutamate, via the activity of different enzymes, involves donation of an amide (γ-nitrogen) group to enable de novo synthesis of nucleotides, amino sugars, and NAD^+ cofactors (**Figures 1**, **4*a***) (**Table 2**). Overexpression of GLS correlates with poor prognosis in patients with glioblastoma, ovarian cancer, breast cancer, and prostate cancer, illustrating the importance of nitrogen donation by glutamine in cancer (16, 39, 60, 61). The ammonia released from GLS/GLS2 and GLUD1/GLUD2 can be incorporated into carbamoyl phosphate in the urea cycle via the function of carbamoyl phosphate synthetase I (CPSI). Carbamoyl phosphate synthetase II (CPSII), by contrast, can accept the amide group from glutamine to generate carbomoyl phosphate, a rate-limiting step in pyrimidine synthesis. Cytidine triphosphate synthetase (CTPS) is responsible for conversion of uridine triphosphate (UTP) into CTP by using the amide group from glutamine. CTPS expression and activity are positively correlated with growth rate in primary liver and kidney carcinomas (62). For purine synthesis, 5-phosphoribosyl-α-pyrophosphate (PRPP), generated from the PPP, is converted into phosphoribosyl-β-amine (PRA) with the addition of an amide group from glutamine by phosphoribosyl pyrophosphate amidotransferase (PPAT) (**Figure 4*a***). Furthermore, the amide group from glutamine can also be transferred to formylglycinamide ribonucleotide (FGAR), which in turn forms phosphoribosylformylglycinamidine (FGAM) for nucleotide synthesis by 5′-phosphoribosylformylglycinamidine synthase (PFAS) (**Figure 4*a***). These studies underscore the importance of glutamine-derived nitrogen in nucleotide precursor synthesis and highlight glutamine's role as a rate-limiting factor in cancer cell proliferation.

3.2. Glutamine as a Nitrogen Donor for Nonessential Amino Acids

NEAAs can be synthesized by healthy cells de novo but may also be derived from dietary sources. However, the high energetic and metabolic demands of cancer cells make them dependent on

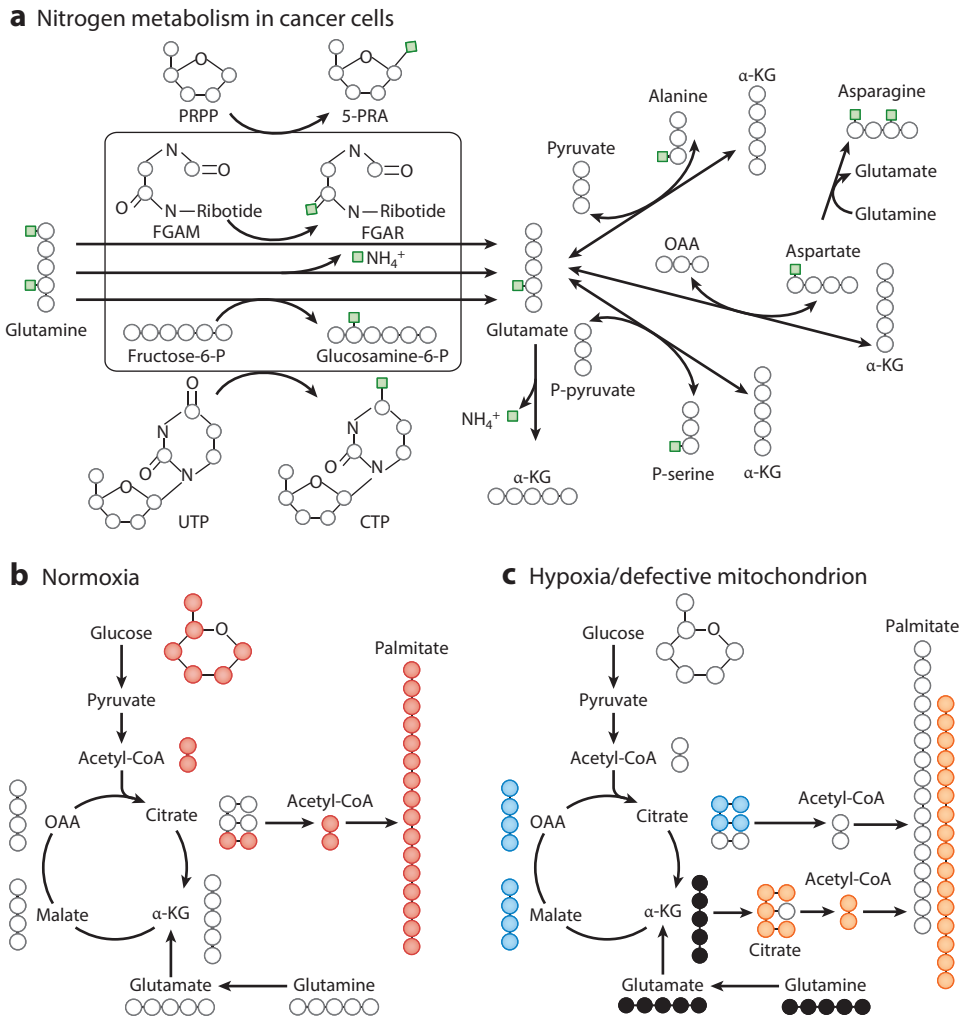


Figure 4

Glutamine provides carbon and nitrogen sources for cells. (*a*) Glutamine donates amide and amino nitrogens for purine, nonessential amino acid, and glucosamine synthesis. The green rectangles represent ^{15}N. (*b*) In normoxia, acetyl-CoA for de novo fatty acids is derived from glucose oxidation by the TCA cycle. Glucose contribution to lipogenesis can be quantified by ^{13}C-labeled tracer experiments. Red circles represent ^{13}C, and unfilled circles represent ^{12}C. (*c*) Tumor cells with defective mitochondria or in hypoxic conditions enhance glutamine-reductive carboxylation for citrate generation to form fatty acids. Black, blue, and orange circles represent ^{13}C, and unfilled blank circles represent ^{12}C. Blue ^{13}C is derived from black ^{13}C-labeled glutamine via glutamine oxidation, whereas orange ^{13}C is derived from black ^{13}C-labeled glutamine through reductive carboxylation. Abbreviations: CTP, cytidine triphosphate; FGAM, 5′-phosphoribosylformylglycinamidine; FGAR, phosphoribosyl-*N*-formylglycineamidine; OAA, oxaloacetate; PRPP, 5-phosphoribosyl-α-pyrophosphate; UTP, uridine triphosphate; α-KG, α-ketoglutarate; 5-PRA, phosphoribosylamine.

Table 2 Carbon and nitrogen contributions of glutamine toward NAD synthesis, aminosugars, purine, pyrimidine, and glutathione

KEGG metabolic pathway	Key metabolite	Number of precursors	Precursor	Carbon contribution	Nitrogen contribution
Nicotinate and nicotinamide metabolism	NAD	1	Nicotinic acid	6	1
		1	PRPP	5	None
		1	ATP	10	5
		1	Glutamine	None	1
			Total	21	7
Aminosugar metabolism	Glucosamine	1	Glucose	6	None
		1	Glutamine	None	1
			Total	6	1
Purine metabolism	IMP	1	PRPP	5	None
		2	N10-formyl-THF	2	None
		1	Bicarbonate	1	None
		1	Aspartate	None	1
		1	Glycine	2	1
		2	Glutamine	None	2
			Total	10	4
Pyrimidine metabolism	UMP	1	PRPP	5	None
		1	Bicarbonate	1	None
		1	Aspartate	3	1
		1	Glutamine	None	1
			Total	9	2
Glutathione metabolism	Glutathione	1	Glutamine/glutamate	5	1
		1	Cysteine	3	1
		1	Glycine	2	1
			Total	10	3

Abbreviations: IMP, inosine monophosphate; KEGG, Kyoto Encyclopedia of Genes and Genomes; N10-formyl-THF, 10-formyl-tetrahydrofolate; PRPP, 5-phosphoribosyl-α-pyrophosphate; UMP, uridine monophosphate.

certain nonessential amino acids. In human pancreatic ductal adenocarcinoma, exogenously provided NEAAs along with α-ketoglutarate can rescue colony formation under glutamine deprivation; however, α-ketoglutarate alone does not have the same effect. This observation indicates that glutamine is a source of NEAAs, with aspartate, alanine, and phosphoserine being the major nitrogen acceptors, as discussed above (**Figure 4*a***). Aspartate is necessary as a nitrogen donor for all nucleotides, and as a carbon donor for UTP and CTP synthesis (**Table 2**) (5). In KRAS-driven cancer cells, aspartate addition can rescue S-phase arrest induced by glutamine deprivation, indicating its critical role in both purine and pyrimidine nucleotide biosynthesis (37). Due to the inherent inefficiency of the aspartate transporter in mammalian cells, the majority of intracellular aspartate is generated from glutaminolysis (63–66). Overexpression of SLC1A3 (a glutamate–aspartate transporter) can rescue proliferation in Jurkat cells with a dysfunctional electron transport chain (ETC) (66).

PFAS: phosphoribosylformylglycinamidine synthase

ETC: electron transport chain

In addition to aspartate, alanine and phosphoserine can be generated via the enzymes GPT and PSAT by use of nitrogens provided by glutamine (**Figure 4*a***) (67, 68). An analysis of biopsied

tumors showed a positive relationship between prognosis in prostate cancer (patient survival) and intracellular alanine concentrations (69). Moreover, a direct comparison of metabolic differences between proliferating and quiescent cells by organotypic three-dimensional models shows that proliferating cells preferentially catabolize glutamate through transaminases for NEAA synthesis (70). Growth arrest in vitro and in vivo induces marked overexpression of GLUD and repression of transaminase activity (especially PSAT), decoupling glutamine anaplerosis and NEAA synthesis.

Asparagine synthetase (ASNS) can also transfer an amide group from glutamine to asparate to generate asparagine (**Figure 4*a***). Asparagine is important for the survival of leukemic cells (71). Reduction in asparagine levels through administration of L-asparaginase has been a widely applied treatment for acute lymphoblastic leukemia (72). A protective role of asparagine in tumor cells has also been demonstrated in glioblastoma under conditions of glutamine deprivation or when intracellular citrate synthesis is inhibited. Furthermore, asparagine can support cyclic AMP–dependent transcription factor (ATF)-dependent adaptive stress responses while repressing cell death induced by ER stress (73).

Glutamine can also be converted into proline and ornithine and can stimulate ornithine decarboxylase (ODC) to activate polyamine synthesis (74). Consequently, a lack of glutamine hinders intestinal epithelial heat shock responses, which can be restored by supplementation of ornithine or polyamines (75).

Furthermore, glutamine is involved in the hexosamine biosynthetic pathway, which is critical for glycopolymer synthesis. Glucosamine fructose-6-phosphate amidotransferase (GFAT) regulates the hexosamine pathway by transferring an amide group from glutamine to fructose-6-phosphate. In hematopoietic cells, IL-3-dependent glutamine uptake is coupled with glucose metabolism to maintain cell growth and survival (54). The NAD synthesis pathway also requires glutamine as an amide group donor for amidation of the nicotinic acid moiety (**Figure 1**) (76).

ASNS: asparagine synthetase

ATF: cyclic AMP (cAMP)-dependent transcription factor

ODC: ornithine decarboxylase

GFAT: glucosamine-fructose-6-phosphate aminotransferase

NNT: NAD(P) transhydrogenase

FADH2: flavin adenine dinucleotide

3.3. Glutamine as a Carbon Donor

Cells utilize endogenous and exogenous sources of lipids to form the lipid membranes required for cell division. Inhibition of fatty acid synthesis hinders tumor formation in xenograft models, highlighting the importance of de novo lipid synthesis in tumor growth (77). In the first step of fatty acid synthesis, acetyl-CoA is carboxylated into malonyl-CoA in the cytosol. The majority of cytoplasmic acetyl-CoA is generated from citrate exported out of the mitochondria, which is metabolized to acetyl-CoA and OAA through ACLY. In normoxia, glucose contributes to most of the acetyl-CoA pool meant for de novo synthesis of fatty acids (**Figure 4*b***). However, under conditions of hypoxia or defective mitochondria or during the formation of anchorage-independent tumor spheroids, cancer cells shift from glucose and glutamine oxidation to reductive carboxylation of glutamine in order to maintain citrate levels (**Figure 4*c***) (78–80). In these circumstances, glutamine directly supplies carbons for citrate production and fatty acid synthesis (78, 81, 82). Glutamine-dependent reductive carboxylation is triggered either by a change in the ratio of α-ketoglutarate to citrate levels or because of the overexpression of NAD(P) transhydrogenase (NNT), which transfers a proton from NADH to $NADP^+$ to form NADPH so as to drive the activity of IDH (83, 84).

3.4. Glutamine, the Electron Transport Chain, and Reactive Oxygen Species

Oxidization of glutamine in the TCA cycle generates one $FADH_2$ (flavin adenine dinucleotide) and three NADH molecules via GLUD, OGDH, SDH, and MDH (malate dehydrogenase). NADH and $FADH_2$ enable the ETC to create the electrochemical gradient required for ATP production.

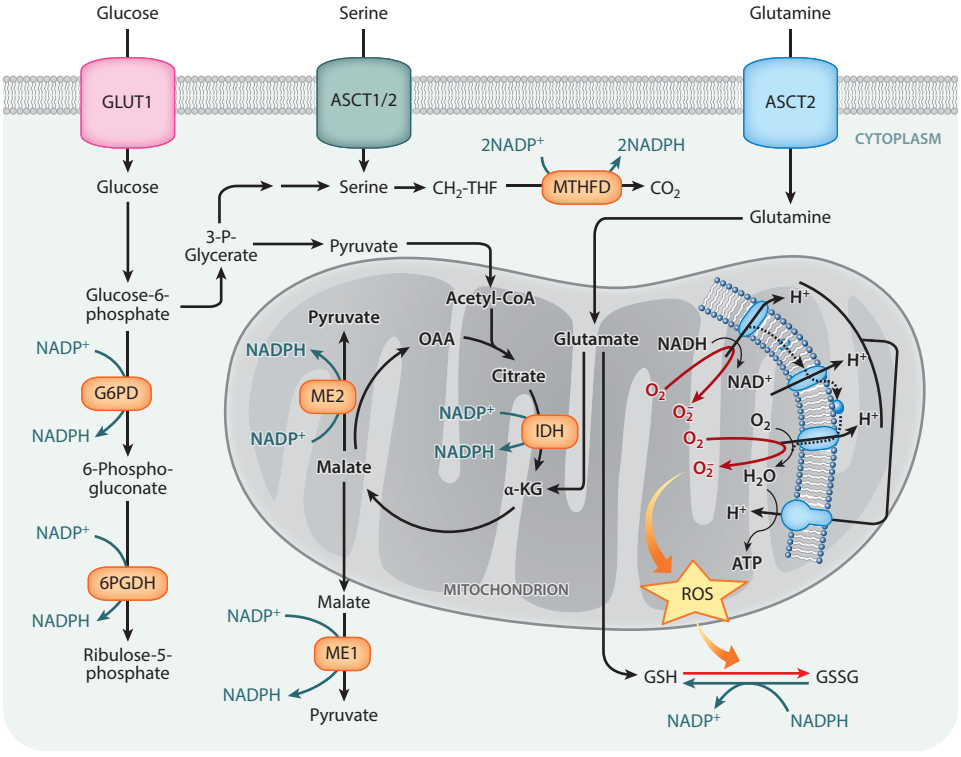


Figure 5

Metabolic pathways control NADPH and ROS balance. Glucose enters the pentose phosphate pathway to generate two NADPH molecules via G6PD and 6PGDH. Serine derived from 3-phosphate glycerate or the microenvironment can assist NADPH generation through the one-carbon metabolism pathway. Glutamine-derived malate generates NADPH through ME1/2. IDH1/2 convert citrate to α-ketoglutarate along with NADPH generation. Through the electron transport chain, O_2^- is generated, leading to increased levels of ROS. ROS convert GSH into GSSG, which can be reduced back to GSH by NADPH. Abbreviations: GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; G6PD, glucose-6-phosphate dehydrogenase; IDH, isocitrate dehydrogenase; ME, malic enzyme; MTHFD, methylene tetrahydrofolate dehydrogenase; OAA, oxaloacetate; ROS, reactive oxygen species; THF, tetrahydrofolate; α-KG, α-ketoglutarate; 6PGDH, 6-phosphogluconate dehydrogenase.

Accordingly, glutamine can enhance the oxygen consumption rate and ATP production in KRAS-mutant cells to promote tumorigenesis (85). In mouse kidney epithelial (iBMK) parental cells or transformed cells after Ras and Akt activation, glutamine drives 60% of the total NADH and $FADH_2$ production, whereas glucose accounts for only 30% (15). Surprisingly, a similar configuration of ATP production is found in hypoxic conditions, highlighting the importance of glutamine in ATP synthesis in cancer cells under various conditions (15). After NADH transfers an electron through the ETC, an incomplete reduction of oxygen generates a superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and a hydroxyl radical (OH•), collectively known as ROS (**Figure 5**). A highly oxidative intracellular environment causes DNA damage, protein denaturation, and lipid peroxidation. However, a certain amount of ROS is necessary for tumorigenesis because ROS induce chromosomal instability and aneuploidy (86).

To mitigate the toxicity of high oxidative stress, a myriad of antioxidants are activated by NADPH, generated primarily via three metabolic pathways (87). The PPP shunts glucose into

ribulose-5-phosphate, the precursor of ribose-5-phosphate for nucleotide synthesis, and generates two NADPH molecules per glucose-6-phosphate molecule (**Figure 5**). In KRAS-transformed cancer cells, the PPP is essential for the support of anchorage-independent growth (88). Accumulating evidence shows that folate-dependent NADPH production is also crucial to maintain the ratio of NADPH/$NADP^+$. Methylene tetrahydrofolate dehydrogenase 1/2 (MTHFD1/2) reactions generate two NADPH molecules along with complete oxidation of one methylene tetrahydrofolate molecule (**Figure 5**) (89). Another important NADPH source is the reaction catalyzed by ME in which malate is decarboxylated to pyruvate and, at the same time, $NADP^+$ is reduced to NADPH (**Figure 5**). Knockdown of ME1 significantly lowers cellular NADPH/$NADP^+$ in conjunction with clonogenic survival of pancreatic ductal adenocarcinoma cells (64, 90). To distinguish the contribution of NADPH from those of these distinct pathways, Fan et al. (87) incubated HEK293T cells with a medium containing the deuterium tracer 3-^{2}H glucose, [2,3,3-^{2}H] serine, or [2,3,3,4,4-^{2}H] glutamine. They found that the PPP, metabolism of one carbon, and ME account for around 30%, 40%, and 30% of overall $NADP^+$ reduction, respectively. In addition to these pathways, mitochondrial ROS are mitigated by NADPH produced by an IDH1-driven reductive carboxylation reaction of glutamine in anchorage-independent growth conditions (91).

NADPH is also required for fatty acid synthesis. In de novo synthesis of lipids, production of 1 molecule of palmitate requires 14 molecules of NADPH. In a reverse process, LKB1/AMPK activation under energy stress reduces NADPH consumption by repressing fatty acid synthesis and enhancing NADPH generation from the oxidation of preexisting fatty acids to maintain ROS homeostasis (92). This mechanism rescues cells from programmed cell death in energy-stressed conditions such as low glucose availability.

Among the antioxidants that can eliminate H_2O_2, reduced glutathione is the most abundant. GSH can be regenerated from its oxidized form, GSSG, along with the conversion of NADPH to $NADP^+$ (**Figure 5**). GSH is a tripeptide; to generate GSH, γ-glutamylcysteine is first produced from L-glutamate and cysteine via the enzyme GCLC. Then glycine is added at the C terminus of γ-glutamylcysteine by the enzyme GSS. Therefore, glutamine functions as both a carbon and a nitrogen donor for GSH synthesis by providing glutamate from the GLS/GLS2 reaction and enabling uptake of cysteine through the x_c^- cystine/glutamate antiporter (**Table 2**) (93).

MTHFD1/2: methylene tetrahydrofolate dehydrogenase 1/2

LAT1: L-type amino acid transporter 1

GDP: guanosine diphosphate

GTP: guanosine triphosphate

3.5. Glutamine and Cell Signaling

Glutamine's role in the regulation of cellular metabolism in cancer cells is not limited to its ability to provide carbon and nitrogen for macromolecular synthesis. Glutamine also orchestrates intracellular signaling to promote tumor growth. The export of glutamine out of the cytoplasm by the heterodimeric bidirectional antiporter LAT1 (L-type amino acid transporter 1) allows the uptake of the essential amino acid leucine (**Figure 6**). Leucine activates mTORC1 protein kinase, which works in conjunction with other stimuli to regulate protein synthesis, ribosomal biogenesis, and autophagy (94–97). This regulation is achieved when leucine binds with Sestrin2 to disrupt the Sestrin2–GATOR2 interaction, which inhibits mTORC1 signaling (**Figure 6**). Additionally, leucine directly binds to GLUD to promote glutaminolysis (98). Overexpression of GLUD leads to mTOR hyperactivation, and addition of membrane-permeable dimethyl α-ketoglutarate causes mTOR to translocate to the lysosome, an essential step for mTOR activation (99–101). Furthermore, glutaminolysis can promote the loading of the mTOR translocation recruiter RagB GTP because glutamine promotes the conversion of GDP (guanosine diphosphate) to GTP (guanosine triphosphate) (99). By contrast, mTOR is a transcriptional repressor of SIRT4, which can negatively regulate GLUD activity and shut down glutamine anaplerosis (42, 102, 103). Glutamine can also affect cytokine secretion in osteosarcoma cells. Glutamine deprivation in these cells induces

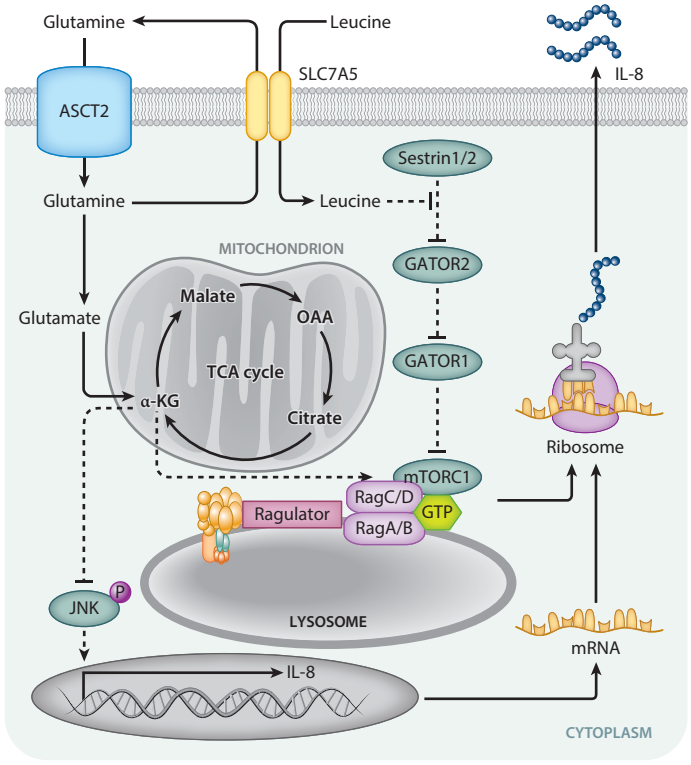


Figure 6

Roles of glutamine in tumor proliferation. Glutamine is taken up by cells via ASCT2 (SLC1A5) and is exported out of the cytoplasm by SLC7A5 to enable uptake of leucine. Leucine binds to Sestrin1/2 and disrupts Sestrin2–GATOR2 interaction. GATOR2 represses GATOR1, an inhibitor of mTORC1. The entry of glutamine into the TCA cycle and conversion into α-KG enhance mTORC1 signaling and repress JNK phosphorylation. Phosphorylation of JNK can induce IL-8 transcription, which is required for IL-8 secretion. Abbreviations: GATOR1/2, GTPase-activating protein toward Rags 1/2; GTP, guanosine triphosphate; IL-8, interleukin-8; JNK, Jun N-terminal kinase; mTORC1, mechanistic target of rapamycin complex 1; TCA, tricarboxylic acid; α-KG, α-ketoglutarate.

an increase in secretion of IL-8 through mTOR/JNK-dependent chemokine secretion (**Figure 6**) (104).

3.6. Glutamine, Apoptosis, and Drug Resistance

Glutamine can hinder cellular apoptosis to induce drug resistance. Acute glutamine deprivation in cells causes apoptosis and cell shrinkage triggered by the CD95-mediated caspase cascade (105, 106). Overexpression of BCL2 (B cell lymphoma 2), a key mediator of the apoptotic pathway, results in a threefold increase of cellular GSH levels. Increased GSH levels can then hinder apoptotic signaling induced by genotoxicity (**Figure 7*a***).

Cisplatin is one of the most effective chemotherapeutic drugs and is the first-line option for treating testicular, bladder, lung, esophagus, stomach, and ovarian cancers. After a series of activation reactions, cisplatin enters the nuclei of proliferating cells to bind with nucleophilic N7 sites of purine bases. Cisplatin can also be toxic to cells if it accumulates in the mitochondria and/or forms adducts with DNA and proteins that trigger cell death. Cisplatin thus causes cell cycle arrest

IL-8: interleukin-8

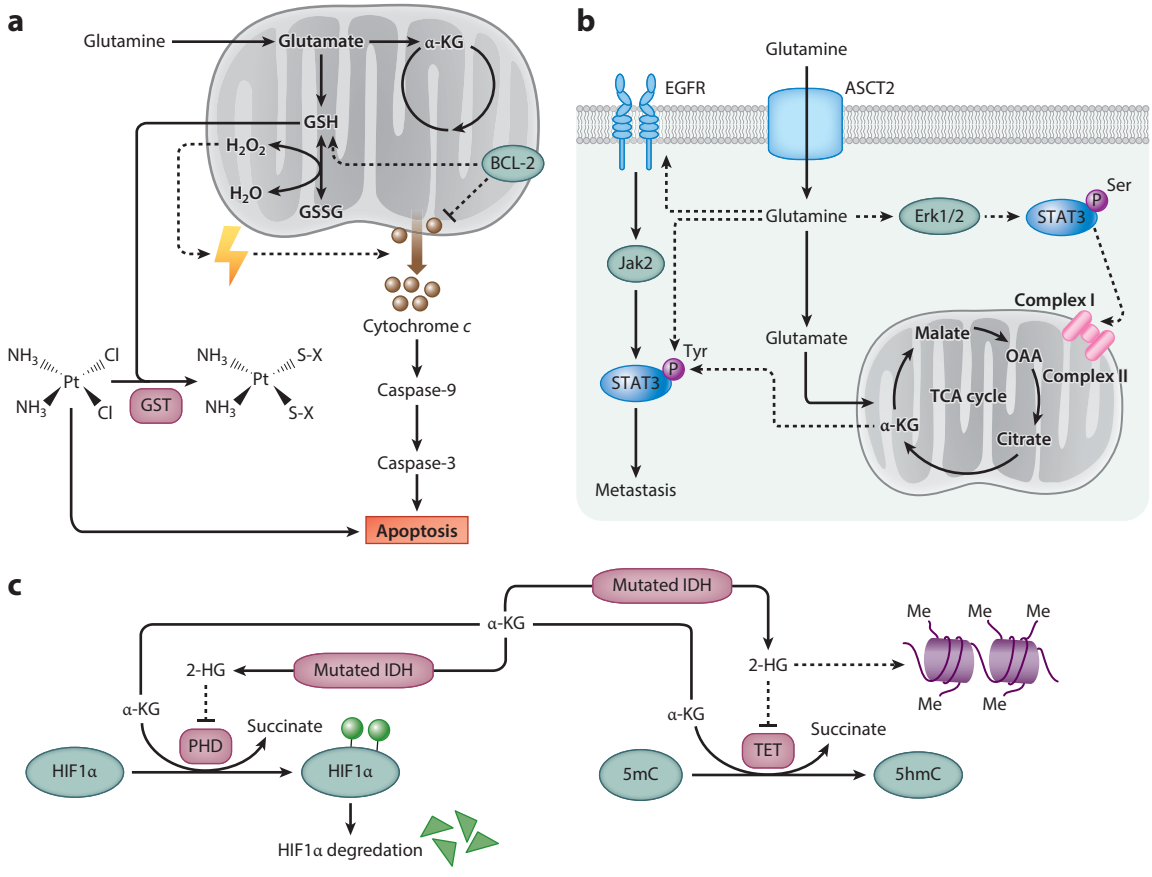


Figure 7

Roles of glutamine in the regulation of tumor metastasis, apoptosis, and epigenetics. (*a*) ROS activate cytochrome *c* release from mitochondria, which in turn trigger the caspase apoptotic pathway. BCL2 enhances GSH synthesis to reduce intracellular ROS and blocks cytochrome *c* release. Cisplatin [$Pt(NH_3)_2Cl_2$] addition induces apoptosis, whereas GSH inactivates cisplatin. (*b*) Glutamine absorbed by cells can activate Erk1/2 and STAT3 serine phosphorylation, which enhances the activity of ETC complex I and complex II. The entry of glutamine into the TCA cycle activates STAT3 tyrosine phosphorylation, which transcriptionally regulates genes important for metastasis. (*c*) α-KG-dependent dioxygenases include a family of PHDs, which regulate the activity of HIF1α. α-KG also promotes 5mC demethylation by serving as a cofactor for the DNA demethylating enzyme TET. Mutated IDH converts α-KG into 2-HG, which represses PHD and TET, thereby decreasing HIF1α degradation and enhancing DNA methylation. Abbreviations: BCL2, B cell lymphoma 2; GSH, glutathione; GST, glutathione *S*-transferase; EGFR, epidermal growth factor receptor; Erk, extracellular signal–related kinase; ETC, electron transport chain; HIF1α; hypoxia-inducible factor 1α; IDH, isocitrate dehydrogenase; Jak2, Janus kinase 2; PHD, prolyl hydroxylase enzyme; ROS, reactive oxygen species; STAT3, signal transducer and activator of transcription 3; TCA, tricarboxylic acid; TET, Tet methylcytosine dioxygenase; α-KG, α-ketoglutarate; 2-HG, 2-hydroxylglutarate; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine.

and induces apoptosis (**Figure 7*a***) (107, 108). However, the endogenous nucleophile GSH binds covalently with cisplatin to prevent its binding to DNA, thereby mediating cisplatin detoxification and conferring cisplatin resistance to cells (108). The combination of the γ-glutamylcysteine synthetase inhibitor BSO and cisplatin represses GSH synthesis and leads to enhanced tumor suppression (109). Cancer-associated fibroblasts (CAFs) within the tumor microenvironment secrete GSH and cysteine, which are taken up by cancer cells, rendering them resistant to platinum-based chemotherapy. However, tumor microenvironment–induced drug resistance is disrupted

CAFs: cancer-associated fibroblasts

by effector T cells, which suppress expression of the x_c^- cystine/glutamate antiporter in CAFs through interferon-γ-induced Jak/STAT1 signaling (110). Therefore, glutamine metabolism is critical for both innate and adaptive immunity. Furthermore, inhibition of glutamine metabolism is a potential therapeutic target to reduce the formation and progression of incipient neoplasias and micrometastases and to suppress CAF-mediated chemoresistance.

EMT: epithelial-to-mesenchymal transition

CTCs: circulating tumor cells

STAT3: signal transducer and activator of transcription 3

D-2HG: D(*R*)-2-hydroxyglutarate

3.7. Glutamine, Epithelial-to-Mesenchymal Transition, and Metastasis

Cancer cells, after undergoing epithelial-to-mesenchymal transition (EMT), detach from the basement membrane at the primary tumor site, migrate, and invade through lymphatics and blood vessels. These cells can relocate to another site to form secondary metastatic tumors, which ultimately lead to the death of many patients. During this multistep process of invasion and metastasis, many metabolic changes occur between cancer cells at the primary site and cells that undergo metastasis (111). Circulating tumor cells (CTCs) have higher expression of peroxisome proliferator–activated receptor γ coactivator 1α (PGC-1α) (which mediates mitochondrial biogenesis and respiration) compared with cancer cells at both primary and secondary sites (112). Therefore, silencing of PGC-1α suppresses the potential of invasive breast cancer cells to disseminate and metastasize (112). However, a contradictory report has found that enhancement of mitochondrial complex I activity in breast cancer inhibits metastasis activity by upregulation of autophagy (113). In addition, mitochondrial ROS from ETC overload or partial ETC inhibition promote superoxide-induced tumor metastasis (10). Together, these observations support the hypothesis that a homeostatic redox balance is necessary to maintain the tumor cells' capacity for distant metastasis. Excessive or low levels of intracellular ROS may not permit metastasis.

Our group recently showed that glutamine deprivation results in a decrease of STAT3 phosphorylation at serine 727, which enhances mitochondrial respiration and STAT3 tyrosine phosphorylation in highly aggressive ovarian cancer cells (**Figure 7*b***) (16). The inhibition of constitutive STAT3 phosphorylation represses the expression level of its target genes, which include genes that regulate metastasis. Addition of α-ketoglutarate rescued STAT3 tyrosine phosphorylation, thereby restoring the invasive capacity of these tumor cells (16). Strategies to inhibit glutaminolysis by knocking down GLS or GLUD expression block STAT3-mediated EMT, migration, and invasion in vivo (16, 114).

3.8. Glutamine and Epigenetics

The breakthrough discovery that cytoplasmic and mitochondrial IDH1 and IDH2 are recurrently mutated in many cancers has triggered research to therapeutically target mutated IDH isoforms. More than 70% of intermediate-grade gliomas exhibit the IDH1 arginine 132 mutations (IDH1 R132H is the most common). Other cancers, such as acute myeloid leukemia and chondrosarcomas, bear both IDH1 R132 and IDH2 arginine 140 and arginine 172 mutations (115, 116). Contrary to the notion that IDH mutation results in loss of function of IDH, the mutation surprisingly enables the conversion of α-ketoglutarate into D(*R*)-2-hydroxyglutarate (D-2HG), an oncometabolite that competitively inhibits α-ketoglutarate-dependent dioxygenases (**Figure 7*c***). Two types of enzymes that are inhibited in this manner are DNA demethylases and histone demethylases; their inhibition results in hypermethylation of DNA CpG islands and histone lysine residues, leading to genome-wide epigenetic alterations (79, 117, 118). In contrast, enhanced activity of α-ketoglutarate-dependent DNA demethylases and histone demethylases causes histone and DNA demethylation that enhances pluripotency in embryonic stem cells (119). The by-product of α-ketoglutarate-mediated demethylation is succinate, which inhibits dioxygenase

activity by competitive binding owing to its structural similarity to α-ketoglutarate. Therefore, the ratio of α-ketoglutarate to succinate regulates the activity of α-ketoglutarate-dependent dioxygenases. The loss of succinate dehydrogenase or fumarate hydratase in tumors leads to altered ratios of α-ketoglutarate to succinate that can affect genome-wide methylation (6, 120, 121). Recent studies have found that α-ketoglutarate can also be converted into the L(*S*)-2-hydroxyglutarate (L-2HG) isoform by MDH and lactate dehydrogenase A under hypoxia. L-2HG is also a potent inhibitor of α-ketoglutarate-dependent dioxygenases, resulting in increased histone H3K9 trimethylation (122, 123). α-Ketoglutarate-dependent dioxygenases also include a family of prolyl hydroxylase enzymes, which regulate the stability of the key metabolic modulator, HIF1α (118). Therefore, glutamine metabolism can directly affect the epigenome via regulating the level of α-ketoglutarate.

L-2HG: L(*S*)-2-hydroxyglutarate

FOXO: forkhead box O

GS: glutamine synthetase

4. GLUTAMINE SOURCES

4.1. Intracellular Glutamine Synthesis

The arterial concentration of glutamine ranges from 0.45 to 0.80 mM, as mentioned above (**Table 1**) (18, 23–25). However, glutamine concentrations in cancer tumor microenvironments are substantially lower than in other tissues, possibly because of excessive glutamine consumption by cancer cells coupled with poor vascular tumor perfusion. Mouse brain has an interstitial glutamine concentration of 80 ± 16 μM, one order of magnitude lower than that in cerebrospinal fluid (517 ± 20 μM) and plasma (598 ± 29 μM) (124). Therefore, it seems that formation of large tumors and metastasis could be hindered due to the lack of sufficient extracellular glutamine. However, intrinsic oncogenic signaling and the availability of multiple extracellular sources make tumor growth possible in environments with limited glutamine supply.

PI3K can be activated by a plethora of external stimuli that further activate serine/threonine protein kinase B (PKB) (125, 126). PKB can phosphorylate forkhead box O (FOXO) transcriptional factors, which positively regulate the expression of glutamine synthetase (GS) (**Figures 3**, **8*a***) (127). In addition, oncogenic *Myc* targets thymine DNA glycosylase to promote demethylation of the *GLUL* (the gene that transcribes GS) promoter, thereby upregulating GS expression (128). GATA3, a master regulator of transcriptional factors, can also directly bind to the *GLUL* promoter in breast luminal cancer cells. Overexpression of GATA3 enables glutamine-dependent breast basal cancer cells to rely less on extracellular glutamine for growth and abrogates glutamine deprivation–induced growth arrest (129).

4.2. Autophagy-Derived Glutamine

Autophagy is the process by which cells digest their nonfunctional organelles via lysosomes to salvage metabolites and biosynthetic macromolecules in response to various physiological or pathophysiological conditions. FOXO-induced GS expression (discussed above) can also enhance autophagy and provide supplemental glutamine (127). Under conditions of nutrient deprivation or the absence of growth factors, cells with poor energetics inhibit mTOR signaling by activation of AMPK. Repression of mTOR signaling induces the formation of autophagosomes, which engulf protein aggregates, damaged organelles, lipid droplets, and intracellular pathogens (130–132). This self-eating process can convert cellular waste into building block units, including amino acids required for maintaining cellular metabolism and homeostasis under stressful conditions (**Figure 8*b***). A defect in the autophagic process resulting from the loss of Atg7 renders cells incapable of generating glutamine from lysosomes. As a result, the cells are unable to sustain mitochondrial metabolism during starvation conditions, and cell death is accelerated (133).

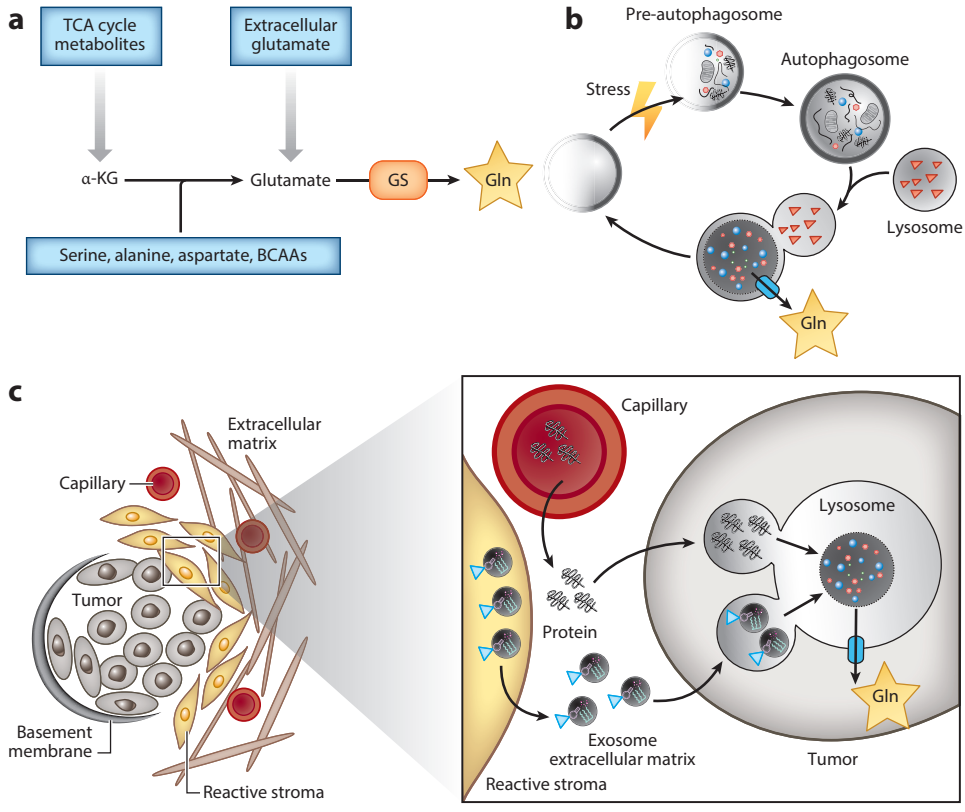


Figure 8

Multiple sources maintain intracellular glutamine levels in cancer cells. (*a*) Cancer cells can generate glutamine through glutamine anabolism. De novo glutamine synthesis is mediated by the intracellular glutamine synthetase enzyme. (*b*) Under nutrient-stressed conditions, cells form autophagosomes, which engulf dysfunctional mitochondria, proteins, and other organelles, and deliver them to lysosomes. (*c*) Cancer cells can take up exosomes secreted by other surrounding cells, as well as proteins from the microenvironment through endocytosis and macropinocytosis. Nutrients from exosomes' metabolite cargo are delivered to lysosomes. Lysosomes contain abundant enzymes that can metabolize nutrients into small molecules and metabolic intermediates. Abbreviations: BCAAs, branched-chain amino acids; Gln, glutamine; GS, glutamine synthetase.

4.3. Extracellular Glutamine Sources

Ras-transformed cells can scavenge unsaturated fatty acids from extracellular lysophospholipids, resulting in levels of intracellular fatty acids that are fourfold higher than in cells in a lysophospholipid-free medium (134). Similarly, cancer cells can take up extracellular protein through macropinocytosis, an endocytic process in which extracellular nutrients are absorbed and internalized through large vesicles (**Figure 8***c*) (135). This process is also regulated by Ras- and Src-driven cellular structural remodeling (135). Because of highly abundant soluble protein (2%) in the plasma and tissue interstitial fluid, macropinocytosis becomes a significant mechanism through which cells obtain nutrients, especially under nutrient-deprivation conditions. In cancer cells cultured in a medium containing subphysiological levels of glutamine, ^{13}C-labeled protein contributed to 10% of intracellular glutamine and other TCA cycle metabolites (135). In this process, extracellular protein is internalized and degraded by lysosomal enzymes to provide

biomass precursors and metabolic intermediates. The addition of bafilomycin A1, an inhibitor of lysosomal vacuolar-type H^+ ATPase, blocked the metabolite-replenishing effect of extracellular protein.

In addition to macropinocytosis, other forms of endocytosis can directly supply nutrients to cancer cells. Exosomes, which are vesicles secreted by cells, can act as metabolite cargo to support prostate cancer growth under low-nutrient conditions via a KRAS-independent mechanism (**Figure 8*c***) (136). As tumors develop, cancer cells recruit nonmalignant stromal cells in their vicinity to form a highly interactive tumor microenvironment (137). These reactive stromal cells coevolve and interact with cancer cells to become an integral part of their physiology. Recent studies have shown that alterations in metabolic phenotypes in stromal CAFs are mediated by cytokines, extracellular acidification by lactate, and ROS derived from tumor epithelial cells. High levels of ROS generated from cancer epithelial cells transform the metabolic state of fibroblasts into a catabolic phenotype, which provides cancer cells with energy-rich metabolites, such as lactate, ketones, and free fatty acids, to enable their proliferation and metastasis via EMT (138, 139). Recently, our group showed that CAFs can directly supply glutamine through the upregulated glutamine anabolic pathway to support ovarian cancer growth under glutamine deprivation conditions (140). Therefore, targeting stromal GS is a viable synthetic lethal approach to the therapeutic treatment of many cancers.

PET: positron emission tomography

FDG: 2-[^{18}F]fluoro-2-deoxy-D-glucose

5. ASSESSING GLUTAMINE UPTAKE AND METABOLISM IN VIVO

5.1. Positron Emission Tomography Imaging of Glutamine Uptake

Positron emission tomography (PET) is a key tool for assessing cancer metabolism in vivo and has extensive clinical implications. PET imaging is based on the synthesis of ligands labeled with radionuclides such as ^{11}C or ^{18}F. These ligands can then be injected into animal models or human subjects in subphysiologic concentrations and can be detected with high sensitivity and specificity in vivo. The radionuclide undergoes a process termed annihilation, which results in the generation of positrons that can be detected in a PET scanner. An important example of PET imaging that is pertinent to cancer metabolism is in the assessment of glucose uptake using an ^{18}F-labeled glucose analog, 2-[^{18}F]fluoro-2-deoxy-D-glucose (FDG). FDG is taken up by cells via glucose transporters; it is then phosphorylated and accumulates in cancer cells at rates proportional to normal glucose uptake (140–143). FDG PET imaging is now routinely used in clinical settings across the world to evaluate glucose uptake as a surrogate for the Warburg effect in the detection and monitoring of cancer.

Similar to FDG, glutamine can be labeled with radionuclides such as ^{18}F or ^{11}C (144–146). The advantage of ^{18}F labeling is a longer half-life of ~110 min versus the relatively short half-life of ^{11}C of ~20 min. The ^{18}F-labeled 2*S*,4*R* stereoisomer of 4-fluoroglutamine (^{18}F-glutamine) is an analog of glutamine that has been synthesized by the Kung group (144, 145). ^{18}F-glutamine is specifically taken up by tumor cells in animal xenografts (**Figure 9*b***) and can be competed out by excess unlabeled glutamine. In vitro studies have shown that ^{18}F-glutamine is not metabolized in the TCA cycle but is incorporated into proteins (144, 145, 147). Glutamine transporters including SLC1A5, which is overexpressed in many cancers, take up ^{18}F-glutamine (144, 147, 148) (**Figure 9*a***). The biodistribution of ^{18}F-glutamine in animal models is similar to that of ^{3}H-labeled glutamine (144). This finding has prompted the first in-human clinical trial with ^{18}F-glutamine in solid tumors (147). Initial results in gliomas show great promise and demonstrate high ratios of tumor to normal brain (147). Furthermore, chemotherapy and radiation therapy showed reduced ^{18}F-glutamine uptake

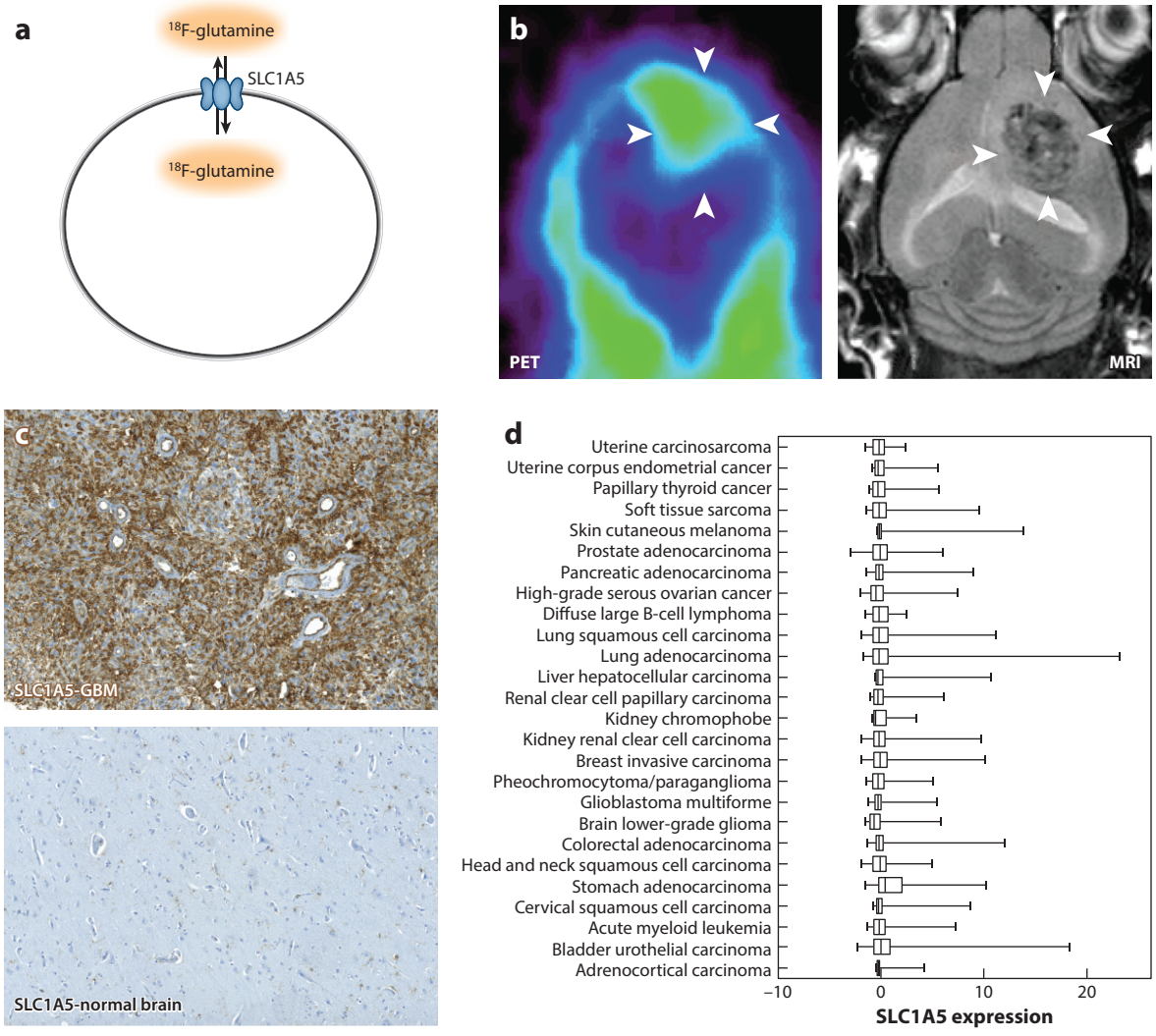


Figure 9

^{18}F-glutamine uptake, positron emission tomography (PET) imaging, and SLC1A5 expression in several cancer. (*a*) ^{18}F-glutamine uptake is mediated mainly by the glutamine transporter SCL1A5 in cancer cells. (*b*) Magnetic resonance imaging (MRI) scan of a glioblastoma (GBM) mouse model (*left*), showing tumor ^{18}F-glutamine uptake (*right*). (*c*) SLC1A5 expression in human GBM and normal brain; expression in GBM is increased. (*d*) SLC1A5 expression levels in various cancers obtained from the Cancer Genome Atlas.

in glioma animal models, suggesting that ^{18}F-glutamine PET imaging could be a potential tool for monitoring therapeutic effectiveness (147).

MRS: magnetic resonance spectroscopy

MRSI: magnetic resonance spectroscopy imaging

5.2. Magnetic Resonance Spectroscopy in Assessing Glutamine Metabolism

Magnetic resonance spectroscopy (MRS) imaging (MRSI) enables detection of various cellular metabolites within tumors and the surrounding microenvironment. This technique takes advantage of specific radio-frequency signals produced by nuclear spins of magnetic resonance–active

nuclei, including ^{1}H, ^{31}P, and ^{13}C (149). ^{1}H-MRSI is a well-established metabolic clinical tool that takes advantage of the increased detection sensitivity and high abundance of ^{1}H within human cancers (150). Techniques such as single-voxel and multivoxel ^{1}H-MRSI enable detection of various metabolic spectra, including those generated by glutamine and glutamate. Because the metabolic spectra of glutamine and glutamate are very similar, they are evaluated collectively (designated as Glx) (149). Glutamate serves as a critical neurotransmitter in the brain and is taken up by astrocytes, metabolized to glutamine, and then recycled back to neurons to generate glutamate via the glutamate/glutamine cycle (151). Therefore, assessment of Glx has been used extensively in both animal models and patients with brain tumors including gliomas, meningiomas, and medulloblastomas to assess tumor invasion, grade, and molecular subtype (152–155).

AICAR: 5-aminoimidazole-4-carboxamide ribotide

5.3. Isotope Labeling of Glutamine in Evaluating Glutamine Metabolism

Whereas ^{1}H-MRSI determines global levels of metabolites, isotope labeling of nutrients such as glucose or glutamine enables detection of the labeled tracer along specific metabolic pathways (156). For example, ^{13}C-labeled glutamine can be used to trace the fate of glutamine carbons in tumor cells (**Figure 4*c***). Similarly, labeling glutamine nitrogens with ^{15}N enables one to follow the fate of glutamine nitrogen metabolism. Many insights have been obtained from isotope-labeled glutamine in cell culture systems by assessing isotope incorporation into downstream metabolites with mass spectroscopy or NMR spectroscopy. However, low levels of isotope-labeled glutamine make its detection in vivo using MRSI very challenging. This challenge can be overcome in ex vivo analyses by rapidly harvesting tumor tissues following injection of isotope-labeled glutamine. The harvested tissues can then be analyzed for incorporation of the isotope along glutamine metabolic pathways by use of mass spectroscopy or NMR spectroscopy. Isotope labeling has allowed us to gain critical insights into glutamine metabolism in vivo. These studies have shown that many factors, such as the specific oncogenic driver and tissue of origin, can influence glutamine metabolism (157). For example, Myc- but not Met-induced liver tumors show TCA cycle–related glutamine metabolism in animal models. In contrast, Myc-driven lung tumors do not show glutamine anaplerosis, but these tumors synthesize glutamine, which is related to the expression of the enzyme GS (157). Furthermore, these techniques have demonstrated differing metabolic adaptations of tumor cells to in vitro versus in vivo conditions. For instance, glioma cells in vitro metabolize glutamine as an anaplerotic substrate in the TCA cycle (20). However, ^{13}C-labeled glutamine in glioma human orthotropic animal models, and a small number of brain tumor patients showed minimal TCA cycle anaplerosis of glutamine (158, 159). Similarly, Ras-driven lung cancer cells showed glutamine anaplerosis in vitro, but not in vivo, in animal models (160). Moreover, glioma animal models showed de novo glutamine synthesis from glutamate by GS, as observed in Myc-induced lung tumors (157, 158, 161). Glutamine thus synthesized in gliomas contributes toward production of nucleotides including purine synthesis intermediates, 5-aminoimidazole-4-carboxamide ribotide (AICAR), and inosine monophosphate (IMP) (161). These studies highlight two factors that are important for understanding glutamine metabolism in cancers: (*a*) Metabolic adaptations may differ between cell culture systems versus in vivo, and (*b*) oncogenic drivers as well as tissues of origin can influence glutamine metabolism.

5.4. Hyperpolarized Magnetic Resonance Spectroscopy

A significant drawback of isotope-labeled MRS is its low signal, which makes it very difficult to assess animal or human subjects without ex vivo harvesting of tumor tissues. This drawback can be overcome through hyperpolarization, which can enhance the sensitivity of ^{13}C detection by more

DNP: dynamic nuclear polarization

GPNA: L-γ-glutamyl-*p*-nitroanilide

EGCG: epigallocatechin gallate

than 10,000-fold (162–164). In this technique, the ^{13}C-labeled tracer is "hyperpolarized" through exposure to microwaves at extremely low temperatures, in a technique termed dynamic nuclear polarization (DNP), immediately prior to tracer administration and scanning (164). This method changes the Boltzmann distribution of ^{13}C, resulting in enhanced detection sensitivity, and allows detection of dynamic metabolic fluxes in real time (162). In vivo detection of the conversion of hyperpolarized [1-^{13}C] pyruvate to [1-^{13}C] lactate in patients with prostate cancer has recently been reported (162). Hyperpolarized ^{13}C-glutamine ([5-(13)C(1)]glutamine) shows great promise in both in vitro and in vivo liver cancer models (163, 165, 166). Similarly hyperpolarized nitrogen molecules in glutamine [(15)ND(2)-amido-glutamine] could also be useful (167). Although these observations have yet to be translated to the clinic, they hold great promise in noninvasively assessing glutamine metabolism in vivo in patients.

6. THERAPEUTIC APPLICATIONS OF TARGETING GLUTAMINE METABOLISM

Because glutaminolysis plays a critical role in cancer cell metabolism, cell signaling, and cell growth, it has presented potential therapeutic avenues to target many cancers. Benzylserine and L-γ-glutamyl-*p*-nitroanilide (GPNA) inhibit the activity of a facile glutamine transporter, ASCT2, and suppress tumor cell proliferation in vitro and in vivo (168, 169). The emergence of small-molecule inhibitors such as BPTES, CB-839, and compound 968 has led to new avenues of metabolism-targeted drugs that block GLS activity and glutaminolysis (170–172). BPTES and its derivative CB-839 allosterically inhibit the dimer to tetramer transition of GLS (60, 170), and compound 968 treatment represses activity of Rho GTPases, thereby inactivating GLS (173). Preclinical trials of these drugs have shown some promise for metabolic therapies in breast cancer and lymphoma (60, 172). CB-839 is currently in Phase I clinical trials. Epigallocatechin gallate (EGCG), an inhibitor of GLUD, and the transaminase inhibitor AOA hinder glutamine TCA cycle anaplerosis and are effective in reducing tumor proliferation in preclinical studies (174, 175). AG-221, AG-120, and AG-881, which specifically inhibit different isoforms of IDH, were recently developed and are currently being evaluated in multiple clinical studies. With continuing investigations of glutamine and glutamine-related metabolism, novel drugs that are promising to be clinically effective are being discovered. An important consideration in developing such drugs is to consider both in vitro and in vivo metabolic adaptations to glutamine in cancer cells.

7. CONCLUSION AND FUTURE PERSPECTIVES

Uncontrolled chronic tumor growth requires energy and cellular building blocks, for which neoplastic cells must consume and metabolize extracellular nutrients for de novo synthesis of macromolecules, generation of ATP, and maintenance of redox balance. Therefore, a great deal of research has focused on understanding how cancer cells utilize available nutrients to meet these demanding metabolic processes. The discovery of aerobic glycolysis in cancer cells by Warburg in 1920 initiated the exploration of cancer metabolism. Three decades later, the first successful metabolic therapy for cancer was introduced, when Farber et al. (176) used an antifolate drug to suppress folic acid synthesis in the nucleotide metabolism pathway for treating children with leukemia. Almost a century after Warburg's discovery, the field of cancer metabolism has grown exponentially and has offered new ways to fight this disease. In this review, we have discussed the pleiotropic role of glutamine in tumor progression from in vitro, in vivo, and potential clinical applications. Glutamine, being the most abundant amino acid in plasma, has a high consumption rate in different types of tumor cells (15, 37, 60). Additionally, rewired metabolism in tumors

enables glutamine-driven anaplerosis to maintain TCA cycle metabolites and provides carbon and nitrogen for production of the biosynthetic macromolecules required for cell division. Glutamine metabolism is directly and indirectly regulated by oncogenes and tumor suppressor genes. The recent discovery of a mutated IDH gene in tumor cells proves that mutation of enzyme-expressing genes may also modify cells' global epigenome and transcriptome to promote tumorigenesis. In addition to intrinsic reprogramming of cancer metabolism, plasma protein and exosomes from stromal cells support the essential nutritional requirements of cancer cells in nutrient-stressed microenvironments. In light of these discoveries, altered glutamine metabolism is now being studied in the context of whole tumors rather than in cancer cells alone. Furthermore, glutamine metabolism in cancer cells demonstrates differing adaptations in cell culture systems versus the in vivo state. The use of advanced technology such as ^{18}F-glutamine for PET imaging and hyperpolarization has provided us with critical tools to assess glutamine metabolism in vivo. Given the pivotal role of glutamine in tumor progression, more research is required in order to gain a comprehensive and mechanistic understanding of glutamine metabolism between tumor and stromal components in patients. These findings will lead us toward clinically relevant therapeutic interventions with efficacious drugs for treating cancer.

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