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Targeting Glutamine Metabolism for Cancer Treatment

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Abstract

Rapidly proliferating cancer cells require energy and cellular building blocks for their growth and ability to maintain redox balance. Many studies have focused on understanding how cancer cells adapt their nutrient metabolism to meet the high demand of anabolism required for proliferation and maintaining redox balance. Glutamine, the most abundant amino acid in plasma, is a well-known nutrient used by cancer cells to increase proliferation as well as survival under metabolic stress conditions. In this review, we provide an overview of the role of glutamine metabolism in cancer cell survival and growth and highlight the mechanisms by which glutamine metabolism affects cancer cell signaling. Furthermore, we summarize the potential therapeutic approaches of targeting glutamine metabolism for the treatment of numerous types of cancer.

Key Words: Cancer, Glutamine, Anaplerosis, Redox homeostasis

INTRODUCTION

Since the discovery that cancer cells can reprogram glucose metabolism towards aerobic glycolysis instead of oxidative phosphorylation by Warburg in the 1920s, there have been significant advancements in understating cancer metabolism (Vander Heiden *et al*., 2009). Metabolic reprogramming is a hallmark of cancer cells, whereby numerous changes in cellular bioenergetics occur, causing the cells to adapt to a variety of stress conditions (Yoshida, 2015). Cancer cells can orchestrate metabolic reprogramming by altering the uptake and catabolism of nutrients, enabling them to maintain proliferative capacity, conferring resistance to oxidative stress, and promoting the evasion of immune-mediated destruction (Hanahan and Weinberg, 2011). In early studies on cancer metabolism, dysregulated glucose metabolism, also called the "Warburg effect", received much attention as a hallmark of cancer, since the glycolytic pathway produces ATP and metabolic intermediates for cancer cell proliferation. However, glucose only supplies a carbon source for biosynthesis; it cannot supply the amino acids and glutathione that rapidly proliferating cancer cells require for the synthesis of nucleic acids.

Several studies have shown that glutamine is a major nutrient involved in multiple aspects of cancer metabolism (Hensley *et al*., 2013). Glutamine is the most abundant amino acid in the blood and muscle (5) and is largely utilized for energy generation and as a precursor for the biomass required for rapid cancer cell proliferation (Windmueller and Spaeth, 1974). In addition to providing a carbon source, glutamine metabolism also acts as a source of nitrogen for the synthesis of nucleic acids and other amino acids and also participates in the regulation of cellular redox homeostasis through a variety of mechanisms (Altman *et al*., 2016). Therefore, most cancer cells are dependent on glutamine and cannot survive in the absence of exogenous glutamine, which has been termed "glutamine addiction" (Eagle, 1955). In light of the importance of glutamine in cancer cell biology, a comprehensive understanding of glutamine metabolism is important for developing effective therapeutic strategies.

In this review, we summarize the diverse aspects of glutamine metabolism including its role in biosynthetic fluxes, the modulation of signal transduction pathways, and the mitigation of oxidative stress. Finally, we discuss potential cancer therapy targeting approaches based on glutamine metabolism.

ROLE OF GLUTAMINE IN CELLULAR GROWTH AND REDOX HOMEOSTASIS

Glutamine addiction in cancer cells

Enhanced glutamine uptake is mediated by several trans-

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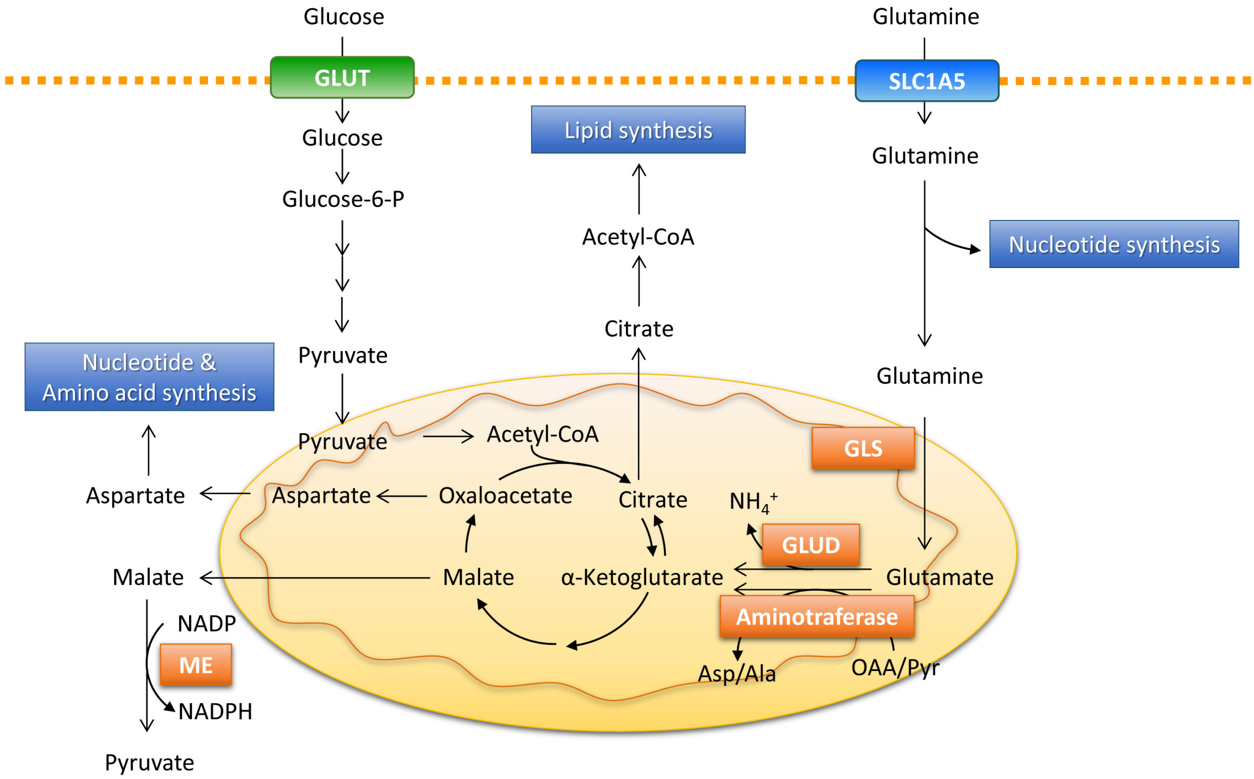

Fig. 1. Glutamine provides a nitrogen and carbon source in biosynthetic pathways. Glutamine enters the cells via the SLC1A5 transporter and contributes to nucleotide biosynthesis directly or is converted to glutamate by GLS. Glutamate is converted to α-ketoglutarate by either GLUD or aminotransferases. Malate from the TCA cycle can be exported to the cytoplasm and converted to pyruvate and generate NAPDH by ME. Oxaloacetate can be converted to aspartate, which supports amino acid and nucleotide synthesis. Glutamine-derived α-ketoglutarate can provide an alternative carbon source for the formation of acetyl-CoA required for lipid synthesis via reductive carboxylation. Glucose-6-P: glucose-6-phosphate, GLS: glutaminase, GLUD: glutamate dehydrogenase, ME: malic enzyme.

porters, including the well-investigated SLC1A5 (also called ASCT2) (Bhutia *et al*., 2015). Many cancer cells, including non-small cell lung cancer (NSCLC), breast cancer, and brain tumor cells have a high dependency on glutamine for their growth and survival and exhibit upregulated SLC1A5 expression (Mohamed *et al*., 2014; Marquez *et al*., 2017). Following the entry of glutamine into the cell via its transporter, the first step of its catabolism occurs through the activation of glutaminase (GLS), which catalyzes the conversion of glutamine to glutamate. Two different isoforms of glutaminase are expressed in mammals, kidney-type glutaminase (GLS1) and liver-type glutaminase (GLS2) (Mates *et al*., 2013). GLS1 is overexpressed in many cancer types and converts glutamine to glutamate, which is then converted to α-KG and channeled into the TCA cycle. In some human cancer tissues, increased levels of GLS1 are associated with a higher disease stage and poor prognosis (Yu *et al*., 2015). On the other hand, the role of GLS2 in cancer is still not completely understood and GLS2 function appears to be context-specific. Some studies have shown that the overexpression of GLS2 reduces the growth of cancer cells, suggesting that GLS2 works as a tumor suppressor and a putative transcription factor (Hu *et al*., 2010). By contrast, other studies have reported that GLS2 in some neuroblastomas is upregulated and contributes to cell survival (Qing *et al*., 2012). The relationship between GLS2 and tumorigenesis is discussed below. In rapidly proliferating cancer cells, glutamine is avidly taken up through a transporter and metabolized by a catalyzing enzyme. Thus, it can provide precursors for energy production and macromolecule biosynthesis, in addition to its ability to regulate cellular signaling.

Glutamine as a source of nitrogen

One of the most important metabolic fates of glutamine is supplying amino and amide nitrogen for biosynthetic pathways, including the production of nonessential amino acids and nucleotides (Fig. 1). Glutamate is converted to α-ketoglutarate by either glutamate dehydrogenase (GLUD), which releases ammonium, or by aminotransferases, which transfers amino nitrogen from glutamate to produce another amino acid (alanine and aspartate) and α-ketoglutarate, without producing ammonia (Yang *et al*., 2017). The aminotransferases glutamate-pyruvate transaminase (GPT), glutamate-oxalate transaminase (GOT), and phosphoserine aminotransferase 1 (PSAT1) catalyze the reversible transfer of amino nitrogen between glutamate to alanine, aspartate, and phosphoserine, respectively (Yang *et al*., 2017). Aspartate contributes to the generation of asparagine through incorporation into urea or is used in nucleotide synthesis. In addition to aspartate, alanine is also used in protein synthesis, but can also be released outside of the cancer cell, carrying some of the excess carbon from glycolysis (DeBerardinis and Cheng, 2010). Phosphoserine is subsequently converted to glycine by serine hydroxymethyltransfer-

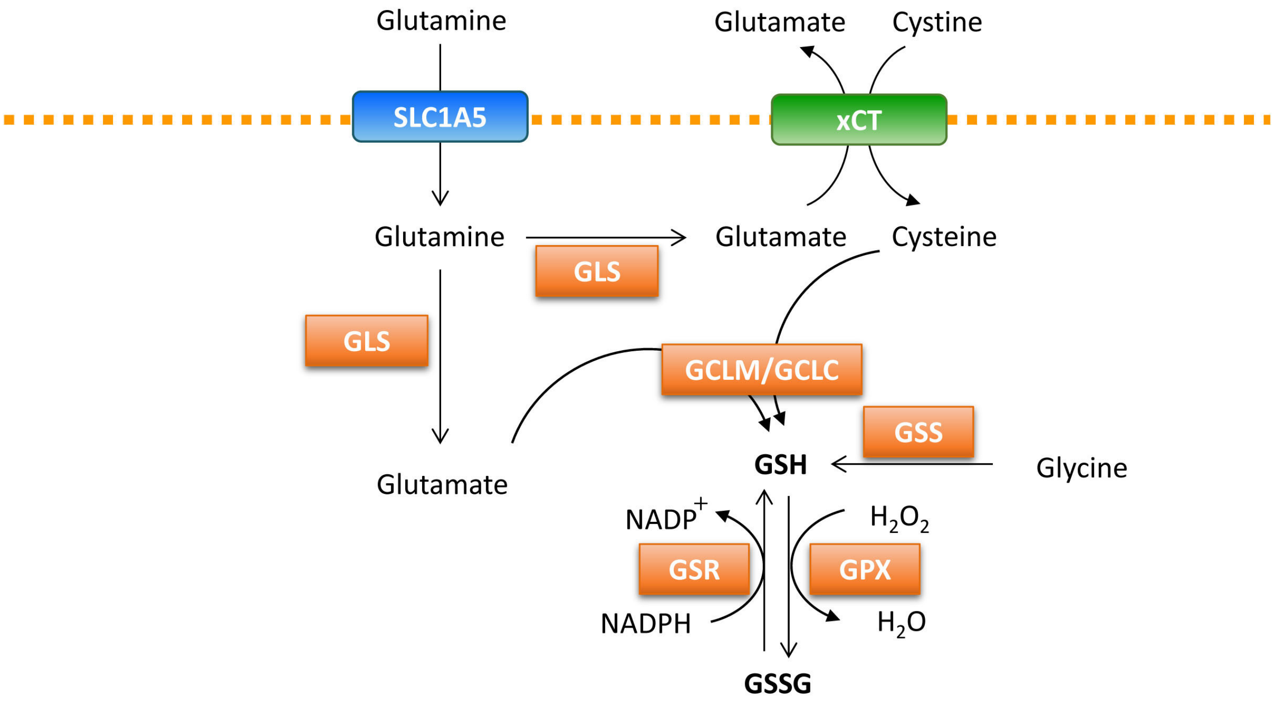

Fig. 2. Glutamine regulates reactive oxidative stress. Glutamine contributes to the generation of GSH, a tripeptide of glutamate, glycine, and cysteine. Glutamate reacts with cysteine to produce GSH via GLCL/GCLC. Glycine is added during the second step of de novo GSH synthesis via GSS. GSH directly eliminates ROS through the action of GPX. NADPH is required for the regeneration of the reduced form of GSH by GSR. GSH: reduced glutathione, GLCL: glutamate-cysteine ligase catalytic subunit, GCLM: glutamate-cysteine ligase modifier subunit, GSS: glutathione synthetase, GPX: glutathione peroxidase, GSR: glutathione reductase, GSSG: oxidized glutathione.

ase, as part of one-carbon metabolism. This integrates cellular nutrients by cycling carbon units from serine inputs to generate diverse outputs, including the biosynthesis of NADPH and nucleotides (Locasale, 2013). A tracer study showed that at least 50% of nonessential amino acids required for protein synthesis arise from glutamine in cancer cells, highlighting the role of glutamine during rapid cellular proliferation (Alberghina and Gaglio, 2014). Glutamine-derived amide nitrogen also contributes to de novo synthesis of purines and pyrimidines, which are required for rapidly proliferating cancer cells (DeBerardinis and Cheng, 2010). Glutamine-derived amide nitrogen units are added to the growing purine and pyrimidine rings, which can explain the observation that *K-RAS*-transformed cells exhibit a delayed transit through S phase under low glutamine conditions, due to a reduced supply of DNA building blocks (Gaglio *et al*., 2009). All of these findings suggest that glutamine serves as a major nitrogen source for amino acid and nucleotide biosynthesis required for cancer cell growth.

Glutamine-derived anaplerosis

Cancer cells require large amounts of lipids as well as nucleotides and amino acids during cell division. Most of the carbon required for fatty acid synthesis in non-proliferating cells comes from glucose, which is converted to acetyl CoA that condensed with oxaloacetate to produce citrate (Vander Heiden *et al*., 2009). During the rapid proliferation of cancer cells, citrate is continuously exported from the mitochondria to the cytosol for lipid biosynthesis. To accommodate this, the replenishment of metabolic intermediates in the TCA cycle, also called anaplerosis, is required. The flux experiments revealed that glutamine, via anaplerotic entry to TCA cycle, replenishes the biosynthetic precursors required for fatty acid synthesis (DeBerardinis *et al*., 2007, 2008). In glioblastoma cells, glutamine-derived oxaloacetate accounts for a high fraction of citrate synthesis, whereas glucose supplies a major carbon source for acetyl-CoA, suggesting that anaplerosis is central to glutamine metabolism (DeBerardinis *et al*., 2007). In addition to citrate synthesis, glutamine provides an alternative carbon source for the formation of acetyl-CoA that is required for lipid synthesis via reductive carboxylation under conditions of hypoxia or mitochondrial dysfunction (Metallo *et al*., 2011; Jiang *et al*., 2016). Tracer experiments demonstrated that 10-25% of lipogenic acetyl-CoA is derived from glutamine via reductive carboxylation in various types of cancer cells (Metallo *et al*., 2011). Glutamine-derived malate is also converted to pyruvate by malic enzyme, which can be metabolized to oxaloacetate or acetyl-CoA for reentry into the TCA cycle (Le *et al*., 2012). Thus, anaplerosis for energy production and fatty acid synthesis in cancer cells is highly dependent on glutamine metabolism, especially under conditions of metabolic stress or oncogenic activation.

Effects of glutamine metabolism on redox homeostasis

Cancer cells are inevitably exposed to more reactive oxygen species (ROS), generated by the mitochondrial electron transport chain during disease progression. When ROS is present in excess, it can damage DNA and other cellular components (Gorrini *et al*., 2013). Cancer cells have several protective mechanisms to avoid death upon excessive ROS exposure. One of the well-known mechanisms is the ability

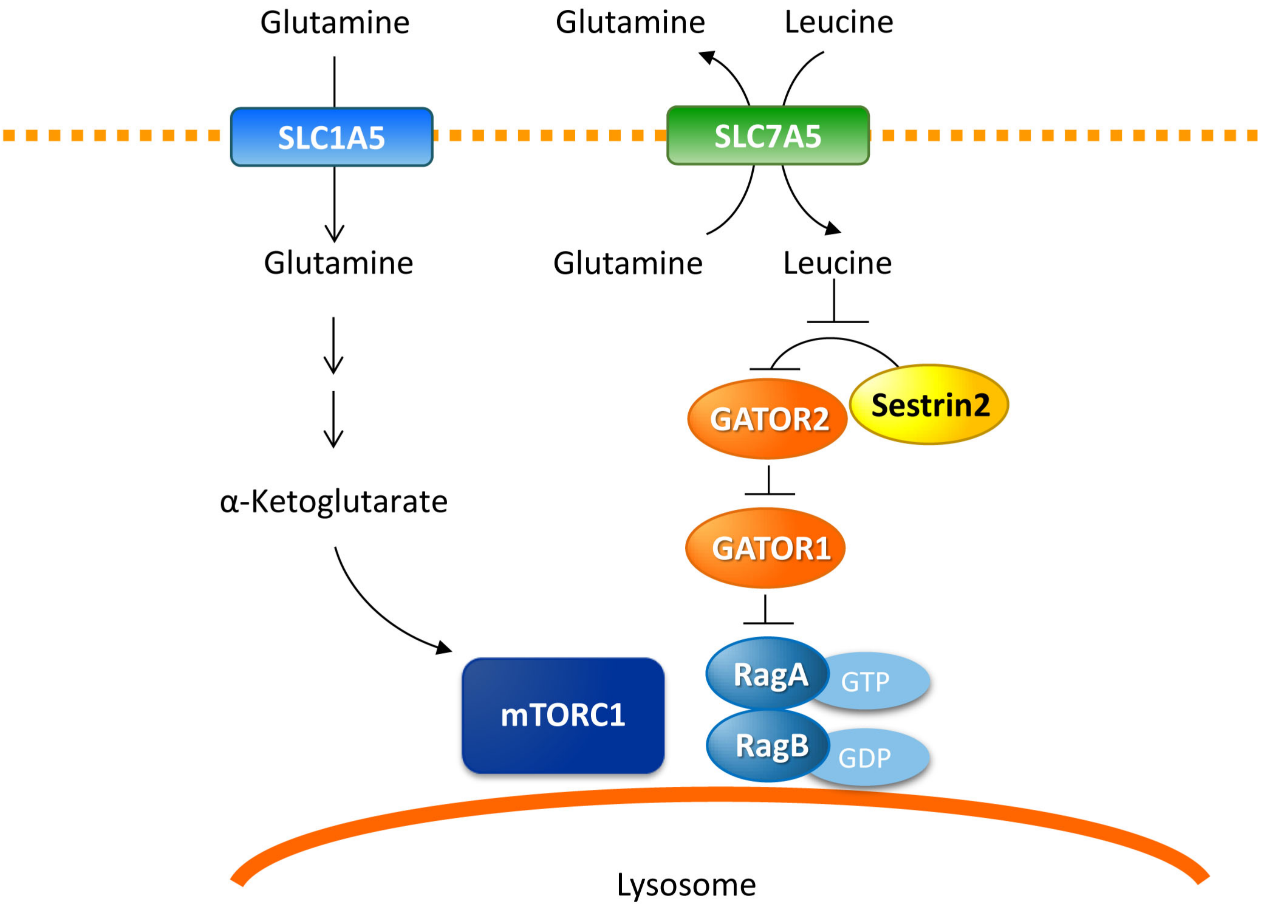

Fig. 3. Glutamine regulates mTORC1 activation. Glutamine activates mTORC1 through the simultaneous efflux of leucine into cells by the bidirectional transporter, SLC7A5. Imported leucine binds to Sestrin2 and disrupts the Sestrin2-GATOR2 interaction, resulting in the recruitment of mTORC1 to lysosomes. Glutamine-derived α-ketoglutarate can directly stimulate lysosomal localization and activation of mTORC1. mTORC1: mechanistic target of the rapamycin complex 1.

of cancer cells to increase antioxidant defense substrates that can lower ROS levels. Glutathione (GSH), a tripeptide of glutamate, glycine, and cysteine, is an abundant endogenous antioxidant molecule that can promote cancer cell survival and redox homeostasis (Fig. 2). Glutamine-derived glutamate and cysteine from the cysteine/glutamate transporter are required for de novo GSH synthesis through the activity of the glutamate-cysteine ligase modifier subunit (GCLM) and the glutamate-cysteine ligase catalytic subunit (GCLC) (Lee *et al*., 2006). Next, glycine is added during the second step of de novo GSH synthesis by glutathione synthetase (GSS) (Lushchak, 2012). GSH acts directly to eliminate hydrogen peroxide through the action of glutathione peroxidase (Lubos *et al*., 2011). GSH can be regenerated from its oxidized form (GSSG), along with the conversion of NADPH to NADP+ (Gorrini *et al*., 2013). The reducing agent NADPH is generated via several mechanisms, including the conversion of malate to pyruvate by malic enzyme as well as through the pentose phosphate pathway and serine/glycine metabolism (Boroughs and DeBerardinis, 2015). Among these, glutamine availability contributes to the production of NADPH by malic enzyme and also participates in the maintenance of redox homeostasis (Son *et al*., 2013). In addition, the IDH1-dependent reductive carboxylation of glutamine generates NADPH, which suppresses mitochondrial ROS in anchorage-independent growth conditions (Jiang *et al*., 2016). The cytosolic reductive carboxylation of glutamine, followed by the import of isocitrate/citrate into the mitochondria can suppress mitochondrial ROS via the generation of NADPH in mitochondria, consequently enabling cells to adapt in anchorage-independent conditions (Jiang *et al*., 2016).

ROLE OF GLUTAMINE ON MODULATION OF SIGNAL TRANSDUCTION

mTORC1 activation

Glutamine coordinates intracellular signaling to promote cancer growth in addition to acting as an important substrate for carbon and nitrogen production. Glutamine regulates mechanistic target of rapamycin complex (mTORC1) activity through several mechanisms (Fig. 3). mTORC1 regulates cell growth and proliferation, which are tightly controlled by multiple signals, including growth factor stimulation, amino acid withdrawal, and hypoxia (Bhaskar and Hay, 2007). Most tumor cells exhibit upregulated mTORC1 activity, which favors tumorigenesis by driving the translation of oncogenic factors, inhibiting autophagy, and enhancing lipid biosynthesis (Zoncu *et al*., 2011). Glutamine activates mTORC1 through the simultaneous efflux of essential amino acids, including leucine, into cells via the bidirectional transporter, SLC7A5/SLC3A2 (van Geldermalsen *et al*., 2016). Imported leucine binds to Sestrin2 and disrupts the Sestrin2-GATOR2 interaction, an inhibitor of mTORC1, leading to the translocation of mTORC1 to lysosomes where Rheb-GTPase enhances mTORC1 activity (Saxton *et al*., 2016; Wolfson *et al*., 2016). Glutamine catabolism results in the production of α-KG and stimulates the lysosomal localization of mTORC1 (Sancak *et al*., 2008; Duran *et al*., 2012). Inhibition of glutaminolysis prevents RagB activation and lysosomal translocation of mTORC1, whereas a cell-permeable α-KG analog stimulates the activation of mTORC1, indicating that mTORC1 activation occurs downstream of glutaminolysis. In our recent study, glutamine deprivation increased Sestrin2 expression in lung and liver cancer cell lines and created a positive feedback loop between Sestrin2 and mTORC2, which is required for the suppression of mTORC1 activity under glutamine-deprived conditions (Byun *et al*., 2017). Thus, by regulating mTORC1, glutamine metabolism participates in modulating multiple cellular signaling pathways.

Glutamine and autophagy

Autophagy is the tightly regulated process by which cells sequester intracellular components in autophagosomes and deliver them to lysosomes, where they are broken down and recycled to provide new building blocks for cell growth in response to extra- or intracellular signals (He and Klionsky, 2009). The role of autophagy in cancer development and progression is context-dependent: while autophagy can suppress the initiation of tumors by removing aberrant proteins and damaged organelles, it also can promote cancer growth by providing substrates for cellular growth and survival in established cancers (White, 2012). Glutamine stimulates mTORC1 activity and in turn, impairs autophagy initiation through the negative regulation of ULK1 by several mechanisms (Hosokawa *et al*., 2009; Jung *et al*., 2009; Nazio *et al*., 2013). In addition, since ROS is an inducer of autophagy, glutamine may also repress autophagy through the elimination of ROS by glutathione and NADPH (Dewaele *et al*., 2010). By contrast, ammonia, generated in catalytic reactions by GLS1 and GLUD, also can act as a signaling molecule, supporting basal autophagy activity in both transformed and non-transformed human cells (Eng *et al*., 2010; Cheong *et al*., 2012). As autophagy provides cellular substrates required for tumor growth, the production of ammonia can reduce cellular stress, and consequently protect cancer cells from death.

ONCOGENE AND TUMOR SUPPRESSORS

KRAS

The oncogenic KRAS mutation is one of the most frequent mutations found in numerous tumor types (Fernandez-Medarde and Santos, 2011). Accumulating evidence supports a molecular link between oncogenic signals, such as KRAS, and glutamine metabolism. Transformed cells harboring high levels of KRAS can orchestrate pleiotropic metabolic reprogramming, including increased glycolytic flux, utilization of glutamine, autophagy, and macropinocytosis (Bryant *et al*., 2014). Flux experiments showed that glutamine supports tumor growth by supplying the increased levels of carbon and nitrogen required for biomass synthesis in KRAS-driven cancer cells (Gaglio *et al*., 2011). More recently, Son *et al*. (2013) demonstrated that oncogenic KRAS altered glutamine metabolism to make it dependent on transaminases, which in turn, supports redox balance due to the conversion of cytosolic aspartate into oxaloacetate, malate, and then pyruvate, simultaneously generating NADPH. Moreover, inhibition of glutamine metabolism increases the sensitivity of pancreatic ductal adenocarcinoma (PDAC) cells to radiotherapy by increasing oxidative stress (Li *et al*., 2015). Thus, cells harboring the oncogenic KRAS are dependent on glutamine for growth and survival, as glutamine provides carbon for biosynthetic pathways and supports the maintenance of redox homeostasis. These findings suggest that targeting KRAS-regulated glutamine metabolism may potentiate the effects of ROS-generating treatments, such as chemotherapy and radiation in PDAC patients.

MYC

Similar to KRAS, MYC-overexpressing cancer cells are often addicted to glutamine, such that glutamine deprivation results in MYC-dependent apoptosis (Yuneva *et al*., 2007). *MYC* coordinates the gene expression that regulates glutamine metabolism at the transcriptional and post transcriptional levels (Gao *et al*., 2009). Interestingly, Gao *et al*. (2009) reported that GLS mRNA levels do not correlate with changes in MYC levels in the human B cell-derived P493-6 cell line or in prostate cancer cells, suggesting that MYC regulates GLS levels post-transcriptionally. In agreement with this notion, *MYC* transcriptionally represses miR-23a/b, leading to higher expression of mitochondrial glutaminase (Gao *et al*., 2009). In addition, MYC appears to selectively bind to the promoter regions of glutamine transporters ASCT2 [SLC1A5] and SN2 [SNAT5], and indirectly induces GLUD (Wise *et al*., 2008; Yuneva *et al*., 2012). Le *et al*. (2012) also found that MYC-inducible human Burkitt lymphoma cells have the ability to survive and even proliferate under hypoxic and glucose-deficient conditions by utilizing the glutamine-driven TCA cycle as an alternative source for energy generation. More recently, MYC-induced reprogramming of glutamine was verified in studies on latent infection of Kaposi's sarcoma-associated herpesvirus and optimal progeny virion generation (Sanchez *et al*., 2015; Thai *et al*., 2015).

p53

p53 is a well-known tumor suppressor that participates in many cellular functions including cell cycle arrest, apoptosis, senescence, and differentiation (Daye and Wellen, 2012). One of the metabolic tumor suppressor functions of p53 is the direct induction of GLS2 expression, which displays an opposing function to GLS1 in tumorigenesis (Hu *et al*., 2010; Suzuki *et al*., 2010). Accumulating evidence suggests that GLS1 and GLS2 have opposite functions in tumorigenesis, although both are involved in the same pathway of catalyzing the conversion of glutamine to glutamate. GLS2 levels in some types of cancers are lower than those in distant and adjacent non-tumor tissues and GLS2 overexpression in cancer cells induces G2/M phase cell cycle arrest (Zhang *et al*., 2013). A previous report has demonstrated that GLS2 is downregulated in glioblastoma cells through DNA hypermethylation, which occurs independently of p53 (Szeliga *et al*., 2016). It is possible that

Table 1. Pharmacological strategies to inhibit glutamine metabolism in cancer cells

Class	Drug	Status	Ongoing clinical trials	
			Cancer type	NCT number
SLC1A5 inhibitor	GPNA γ-FBP Benzylserine	Preclinical tool	-	-
GLS inhibitors	BPTES	Preclinical tool	-	-
	CB-839	Phase I clinical	Hematologic tumors Solid tumors (TNBC, NSCLC, RCC, Mesothelioma…)	NCT02071888 NCT02071862 NCT02771626
	968	Preclinical tool		
GLUD inhibitor	EGCG	Preclinical study	Colorectal Cancer (not yet open for participants recruitment)	NCT02891538
	R162	Preclinical tool	-	-
Aminotransferase inhibitors	AOA	Clinically used to treat tinnitus	-	-

GPNA: Benzylserine and L-γ-gluatamyl-p-nitroanilide, γ-FBP: γ-folate binding protein, GLS: glutaminase, BPTES: bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide, TNBC: Triple-negative Breast Cancer, NSCLC: Non Small Cell Lung Cancer, RCC: Renal Cell Carcinoma, GLUD: glutamate dehydrogenase, EGCG: pigallocatechin gallate, AOA: aminooxyacetate.

the transcription of GLS2 may be controlled by other members of the p53 family, such as p63 and p73 (Giacobbe *et al*., 2013; Velletri *et al*., 2013). More recently, Kuo *et al.* (2016) demonstrated that GLS2 inversely correlated with advanced-stage, vascular invasion, early recurrence and poor prognosis in HCC patients. One possible mechanism that could explain the different roles of GLS2 and GLS1 in tumorigenesis is the non-enzymatic action of GLS2. Several studies have demonstrated that GLS2 suppresses HCC metastasis through the inhibition of snail expression or small GTPase Rac activity, neither of which are related to the glutaminolysis function of GLS2 (Kuo *et al*., 2016; Zhang *et al*., 2016). Given that GLS2 is associated with increased glutathione levels, similarly to GLS1, it is likely that it can also play a role in regulating the oxidative stress-resistant properties of cancer cells. Indeed, some studies have reported that tumor tissues from radio-resistant patients exhibit significantly higher GLS2 levels than those from radio-sensitive patients and that apoptosis in response to radiation is increased in GLS2-knockdown cancer cells (Xiang *et al*., 2013). GLS1 and GLS2 have different structural and kinetic properties and are subject to different regulatory mechanisms (Curthoys and Watford, 1995). Therefore, further study is required to explore the context-dependent divergent effects of GLS2 on tumorigenesis and its regulatory mechanisms in response to external stimuli.

CLINICAL OPPORTUNITIES

Pharmacological strategies to inhibit glutamine metabolism in cancer cells

As previously reviewed, glutamine metabolism exhibits pleiotropic effects on cancer cell signaling and proliferation and therapeutic suppression of glutamine metabolism is considered to be an attractive anticancer strategy (Table 1). Benzylserine and L-γ-glutamyl-p-nitroanilide (GPNA), the inhibitor of the glutamine transporter SLC1A5, have been shown to be effective agents in the treatment of glutamine-dependent cancers (Hassanein *et al*., 2015). However, unless targeted to a precise pathway in tumor cells, these drugs induce toxicity in healthy cells that require glutamine for other pathways. Small molecule inhibitors, such as bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide (BPTES), CB-839, and compound 968, represent a new class of metabolism-targeted drugs that inhibit GLS isoforms not commonly expressed in normal cells (Chen and Cui, 2015; Xiang *et al*., 2015). BPTES, a specific GLS1 inhibitor, suppresses tumor growth *in vitro* and *in vivo* in various cancer cell types. However, BPTES is not a good candidate for GLS inhibition because of its poor solubility and bioavailability (Chen and Cui, 2015). CB839, currently undergoing a phase one clinical trial, is a selective and more potent GLS1 inhibitor than BPTES. CB839 exhibits a significant antitumor effect in triple-negative breast cancer cells and in leukemia cells that require glutamine for their growth (Gross *et al*., 2014; Jacque *et al*., 2015). In contrast to BPTES and CD-839, 968 is a specific inhibitor of GAC, a shorter isoform of the kidney-type glutaminase (Erickson and Cerione, 2010). The effects of 968 were also demonstrated in a variety of cancers including brain, pancreatic, and breast cancer cells that are highly resistant to conventional chemotherapy (Katt *et al*., 2015). Although newly discovered GLS inhibitors such as CB839 and 968 have a higher efficacy and a lower toxicity, the potential side effects of inhibiting glutamine metabolism should be considered (Masson *et al*., 2006; Bunpo *et al*., 2008). Epigallocatechin gallate (EGCG) and R162, an inhibitor of GLUD, as well as aminooxyacetic acid (AOA), a transaminase inhibitor, attenuated tumor growth in preclinical studies by disturbing the anaplerotic use of glutamine in the TCA cycle (Korangath *et al*., 2015).

Key strategies for circumventing therapeutic resistance in cancer

Cancer cells gain their stemness and chemoresistant properties through the upregulation of compensatory pathways when conventional therapy induces metabolic stress (Kim, 2015). As glutamine metabolism contributes to cancer cell proliferation and to the development of adaptation to metabolic stress, the inhibition of glutamine metabolism may be a promising adjuvant strategy to suppress the development of resistance to conventional cancer treatments (Hernandez-Davies *et al*., 2015; Baenke *et al*., 2016). For example, glioblastoma treatment resistance to mTOR inhibitors is likely the result of a compensatory glutamine metabolism mechanism, suggesting that a combined inhibition of GLS1 and mTOR could potentially overcome this type, of resistance (Tanaka *et al*., 2015). In addition, our recent study demonstrated increased glutamine metabolism and reduced glutamine carboxylation in sorafenib-resistant liver cancer cells, where metabolic reprogramming overcame sorafenib resistance (Kim *et al*., 2017). Given that highly invasive and metastatic cancer cells are more dependent on glutamine compared to less invasive cells (Yang *et al*., 2014), combination therapy involving the inhibition of glutamine metabolism could constitute a novel approach to preventing metastasis. Indeed, dual inhibition with BPTES and 5-fluorouracil elicits cell death synergistically through cell cycle arrest, which results in remarkable antitumor effect in a preclinical xenograft model of NSCLC (Lee *et al*., 2016). Collectively, targeting compensatory glutamine metabolism pathways may be a key strategy for circumventing therapeutic resistance in various cancers.

Challenges for clinical use

Despite the significant advances in the understanding of glutamine metabolism in cancer cells, there are still obstacles to overcome in the clinical application of inhibitors of glutamine metabolism pathways. Notably, in the *Kras*-driven lung cancer mouse model, glutamine was not the preferred carbon source for the TCA cycle in a study that utilized isotope-labeled glucose (Davidson *et al*., 2016). This *in vivo* result is in contrast with *in vitro* observations. Moreover, the study using human glioblastoma orthotopic tumors which have metabolic similarities to primary human GBMs showed the accumulated glutamine in tumor tissues is synthesized by de novo from glucose-derived glutamate and minimal glutaminolysis (Marin-Valencia *et al*., 2012). Moreover, high expression of glutamine synthase in cancer cells can promote glutamine-independent growth and resistance to therapies that restrict glutamine metabolism (Hernandez-Davies *et al*., 2015; Baenke *et al*., 2016). As tumors contain numerous cell types that work together to support tumor growth, the metabolic crosstalk between cancer cells and neighboring cells is crucial for the understanding of tumorigenesis. A recent study demonstrated that cancer–associated fibroblasts upregulated the glutamine anabolic pathway to support cancer cell growth. Thus, disrupting metabolic crosstalk between cancer cells and stromal cells by co-targeting stromal glutamine synthetase and cancer cell glutaminase could represent a promising approach to counteract tumor growth (Yang *et al*., 2016). Further investigations are needed to understand how glutamine bioavailability is regulated in the tumor microenvironment and to guide the selection of successful metabolic therapies in the clinic.

CONCLUSION AND FUTURE PERSPECTIVES

During rapid proliferation, cancer cells must optimize metabolic adaptability by balancing nutrient utilization for the synthesis of building blocks, generation of ATP, and maintenance of redox homeostasis. Glutamine metabolism acts as a central player in the regulation of uncontrolled tumor growth by modulating bioenergetic and redox homeostasis and serving as a precursor for biomass synthesis. Intrinsic oncogenic alterations as well as the surrounding tumor microenvironment regulate metabolic reprogramming, resulting in cancer cells that are “addicted” to glutamine metabolism. Although targeting glutamine metabolism pathways represents a promising strategy for the clinical design of therapeutic agents, developing an effective drug has been challenging. Nevertheless, a comprehensive understanding of glutamine metabolism is of the utmost importance, because it provides valuable insights into the pathways that could be targeted for the development of novel therapeutic strategies for the treatment of advanced or drug resistant cancers.

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