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REVIEW

Glutaminolysis as a target for cancer therapy

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Cancer cells display an altered metabolic circuitry that is directly regulated by oncogenic mutations and loss of tumor suppressors. Mounting evidence indicates that altered glutamine metabolism in cancer cells has critical roles in supporting macromolecule biosynthesis, regulating signaling pathways, and maintaining redox homeostasis, all of which contribute to cancer cell proliferation and survival. Thus, intervention in these metabolic processes could provide novel approaches to improve cancer treatment. This review summarizes current findings on the role of glutaminolytic enzymes in human cancers and provides an update on the development of small molecule inhibitors to target glutaminolysis for cancer therapy.
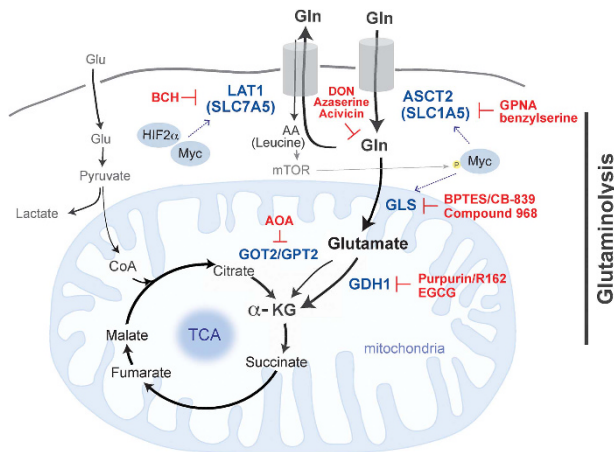
INTRODUCTION

Cancer cells display enhanced and unusual metabolic activities compared with normal differentiated cells, as they reprogram their metabolic machinery in order to satisfy their bioenergetic and biosynthetic requirements.[1] One of these metabolic abnormalities is that cancer cells take up glucose at higher rates than normal tissue, yet use less glucose for oxidative phosphorylation (OXPHOS) and favor the incomplete oxidation of glucose through the glycolytic pathway even in the presence of oxygen (aerobic glycolysis or Warburg effect).[2,3] Pyruvate generated from the glycolytic pathway is converted to lactate, rather than being used in the tricarboxylic acid (TCA) cycle. Although the requirement for mitochondrial ATP production is decreased in tumor cells, the demand for biosynthetic precursors and NADPH is increased.[4] To compensate for these changes and to maintain a functional TCA cycle, cancer cells often rely on elevated glutaminolysis.[5–8] Glutamine, transported into the cells through transporters such as SLC1A5 and SLC7A5, is converted to glutamate and further to alpha-ketoglutarate (α-KG) by glutaminase (GLS), GDH and other enzymes to enable ATP production through the TCA cycle and to provide nitrogen, sulfur and carbon skeletons for producing necessary biosynthetic precursors for growing and proliferating cancer cells (Figure 1).[5–8]

Glutaminolysis, which catabolizes glutamine to generate ATP and lactate, is a metabolic pathway that involves the initial deamination of glutamine by GLS, yielding glutamate and ammonia. Glutamate is then converted to α-KG, a TCA cycle intermediate, to produce both ATP and anabolic carbons for the synthesis of amino acids, nucleotides and lipids (Figure 1).[5] The conversion of glutamate to α-KG is catalyzed by either glutamate dehydrogenase (GDH) or transaminases, such as glutamate pyruvate transaminases (GPTs, that is, alanine aminotransferases) and glutamate oxaloacetate transaminases (GOTs, that is, aspartate aminotransferase), which convert α-keto acids into their corresponding amino acids (Figure 1). Glutamine not only provides a major substrate for respiration but also for the synthesis of other macromolecules, such as nucleotides, proteins and hexosamines.[9,10] In addition to its critical role in providing carbon and nitrogen for macromolecule biosynthesis, glutaminolysis also has an important role in regulating redox balance, mTOR signaling, apoptosis and autophagy.[11–15]

For decades, researchers have shown that glutamine metabolism is also critical for cancer cell growth and survival by supporting macromolecular biosynthesis and maintaining bioenergetics and redox balance.[16–21] High extracellular glutamine concentrations stimulate tumor growth and are essential for cell transformation.[22–24] Glutamine utilization is higher in tumor cells as well as rapidly diving cells including lymphocytes.[25] Moreover, the flux of mitochondrial enzymes involved in glutamine/glutamate oxidation is elevated in tumor cells compared with normal cells.[21,26] Glutaminolysis contributes to tumor growth in two distinct but connected ways, by promoting cell proliferation and inhibiting cell death. The major function of glutaminolysis is to supply intermediary metabolites in the TCA cycle for cell growth. For example, glutaminolysis produces α-KG and replenishes the TCA cycle, which not only provides intermediates for other biosynthetic pathways but also supports energy production.[7] Glutamine is required for nucleotide biosynthesis through donating its nitrogen to purines and pyrimidines. In addition, glutamine contributes to the biosynthesis of hexosamine and certain nonessential amino acids.[20] Thus, glutamine is indispensable for cell proliferation by providing nitrogen and carbon skeletons for macromolecule biosynthesis.[23] In addition to these well-recognized roles, glutamine contributes to sustaining cell proliferation. Recent studies have uncovered several novel functions that account for its roles in regulating cell proliferative events. For instance, cancer cells under hypoxia or with defective mitochondria can use glutamine-derived α-KG to produce citrate through reductive carboxylation, which is critical for lipid synthesis in these cells.[27–29] Glutamine flux is reported to regulate mTOR activation to coordinate cell growth and proliferation.[30] Furthermore, glutaminolysis mediates lysosomal translocation and subsequent activation of mTORC1.[11] Collectively, these findings expand the role of glutaminolysis in metabolic rewiring to support cancer cell proliferation and tumor growth.

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Figure 1. Glutaminolysis in cancer cells. Glutamine is transported through transporters (i.e., SLC1A5 and SLC7A5) to enter the glutaminolysis pathway. Enzymes in the glutaminolysis pathway suggested to be potential anticancer targets are shown in blue and inhibitors of these enzymes are listed in red. Dotted arrows show transcription factors involved in the regulation of relevant enzymes. α-KG, α-ketoglutarate; ASCT2, ASC amino acid transporter 2; AOA, aminooxyacetate; BCH, 2- aminobicyclo(2,2,1)-heptane-2-carboxylic acid; BPTES, bis-2-[5-phenylacetamido-1, 2, 4-thiadiazol-2-yl] ethyl sulfide; DON, 6-diazo-5-oxo-L-norleucine; EGCG, epigallocatechin gallate; GDH1, glutamate dehydrogenase 1; GLS, glutaminase; GOT2, glutamate oxaloacetate transaminase 2; GPNA, γ-L-glutamyl-p-nitroanilide; GPT2, glutamate pyruvate transaminase 2; LAT1, L-type amino acid transporter 1.

Equally important, glutaminolysis is also involved in many metabolic processes and signaling pathways that inhibit cell death. Cancer cells constantly encounter a variety of stress signals *in vivo* such as reactive oxygen species (ROS) and nutrient limitations. Consequently, cancer cells must rewire their metabolic pathways under these environmental conditions. For example, mounting evidence suggests that cancer cells show elevated levels of ROS compared with normal cells. A moderate increase in ROS promotes cell proliferation and differentiation, whereas excessive amounts of ROS inevitably cause oxidative damage to proteins, lipid and nucleotides.[31,32] Glutaminolysis is directly involved in maintaining ROS homeostasis, which is crucial for cell growth and survival. Glutamine supports the production of glutathione (GSH) and nicotinamide adenine dinucleotide phosphate (NADPH), which are two major reducing powers in the cell.[33,34] In addition, glutamine-derived fumarate has been shown to be important for the control of oxidative stress through several distinct mechanisms including upregulating the activity of ROS scavenging enzyme glutathione peroxidase 1 (GPx1) and activating Nrf2 antioxidant signaling.[12,35,36] The diverse contributions of glutaminolysis to redox homeostasis highlight the complexity of metabolic rewiring in response to environmental cues.

In this review article, we highlight recent advances in our understanding of glutaminolytic enzymes in regulating cancer cell metabolism, cell signaling, and redox homeostasis. Furthermore, we will discuss their potential therapeutic application for treating human cancers.

GLUTAMINE TRANSPORTER

Mammalian cells are able to synthesize glutamine through glutamine synthetase (GLUL), which renders exogenous glutamine dispensable. However, GLUL is not highly expressed in all tissues.[23] Moreover, glutamine becomes conditionally essential when the demand for glutamine surpasses the supply, especially for rapidly proliferating cells such as cancer cells.[37] Glutamine

uptake is mediated through membrane-anchored amino acid transporters.[38] Mammalian cells express more than two-dozen amino acid transporters, the expression of which is tightly controlled in a tissue-specific manner.[38] Many of the transporters are overexpressed in cancer cells and it has been reported that certain oncogenes or tumor suppressors regulate their expression. For example, SLC1A5 (ASCT2), a Na$^+$-coupled transporter for glutamine, alanine, serine and cysteine, is upregulated by Myc[33] and downregulated by retinoblastoma protein (Rb).[39] Also, SLC7A5 (LAT1), a bidirectional transporter that regulates simultaneous efflux of glutamine out of cells and influx of leucine into cells, is upregulated by HIF-2α and Myc, and is highly expressed in renal cell carcinoma and prostate cancer.[33,40] These observations suggest the existence of a functional link between oncogenes and glutamine uptake that promotes glutaminolysis and tumor growth. Indeed, Nicklin et al.[30] reported that SLC1A5 is coupled with SLC7A5 to activate mTOR signaling. Glutamine is transported into the cells through SLC1A5 and subsequently the efflux of glutamine out of the cells via SLC7A5 is coupled to the entry of leucine, which activates mTOR signaling and coordinates cell proliferation and growth.[30] Several studies further demonstrate that glutamine transporters, such as SLC1A5 and SLC7A5, are required for the growth of a variety of tumors including acute myeloid leukemia (AML),[41] lung cancer,[42,43] kidney cancer[40] and melanoma.[44] In addition, targeting SLC1A5 by either RNAi or small molecule inhibitors, benzylserine and L-γ-glutamyl-p-nitroanilide, suppresses cancer cell proliferation *in vitro* and tumor growth *in vivo*.[42,43] More importantly, the expression of glutamine transporters inversely correlates with the prognosis of cancer patients, indicating the potential of glutamine transporters as a prognostic biomarker for cancer treatment.

GLUTAMINASE

Glutaminolysis starts with the conversion of glutamine to glutamate, which is catalyzed by the GLSs in mitochondria.[45] There are two forms of GLS in humans: kidney-type glutaminase (GLS, KGA or GAC) encoded by *GLS* and liver-type glutaminase (GLS2, LGA or GAB) encoded by *GLS2*. GLS is expressed ubiquitously, whereas GLS2 is expressed primarily in the liver.[46] Previous studies have shown that GLS is expressed in a wide variety of tumors and its upregulation correlates with tumor growth.[46] However, the regulation of GLS in cancer cells is not well understood. A few studies indicate a functional link between *MYC*, an oncogene, and glutamine metabolism. Yuneva et al.[47] reported that depletion of glutamine induces apoptosis that depends on activity of ectopic MYC. Wise et al.[48] and Gao et al.[33] reported that Myc induces a transcriptional program that promotes glutaminolysis in cancer cells. Further metabolomic studies confirm that glutaminolysis is profoundly affected by induction of Myc.[49,50] Moreover, it has been shown that the mTOR pathway upregulates GLS expression through enhancing translation of c-Myc protein.[51] mTOR signaling stimulates glutaminolysis by not only upregulating GLS but also enhancing the activity of another glutaminolytic enzyme, glutamate dehydrogenase 1 (GDH1),[52] which indicates the complexity of the crosstalk between glutaminolysis and mTOR signaling.

In addition to its transcriptional regulation by c-Myc, GLS can be regulated through the modulation of its enzymatic activity or protein stability.[53–55] NF-κB[53] or Raf-MEK-ERK signaling[54] regulates GLS activity without affecting its protein expression. Colombo et al.[55] reported that GLS is targeted for destruction by APC/C-Cdh1 in a cell cycle-dependent fashion. Moncada et al. reported that APC/C (anaphase-promoting complex/cyclosome)-Cdh1, the ubiquitin ligase that controls the G1 to S phase transition, regulates GLS1 and the glycolytic enzyme PFKFB3. Decreased APC/C-Cdh1 in G1 phase stabilizes both GLS1 and PFKFB3, providing a proliferative advantage to cancer cells.[56] Collectively,

these findings suggest that the activity of GLS is regulated by diverse oncogenes and survival signals in cancer cells.

GLS can be inhibited using RNAi or small molecule inhibitors such as bis-2-[5-phenylacetamido-1, 2, 4-thiadiazol-2-yl] ethyl sulfide (BPTES),[57] CB-839[58] and compound 968.[53] Inhibition of GLS has been shown to significantly suppress tumor growth in several experimental models including breast cancer and lymphoma.[33,53] Reduction of GLS expression by RNAi decreases ATP level and increases ROS level. Both the TCA cycle metabolite oxaloacetate (OAA) and ROS scavenger N-acetylcysteine (NAC) partially rescue the decreased proliferation of cancer cells induced by GLS silencing.[33] This finding emphasizes the significance of glutaminolysis in replenishing TCA cycle intermediates and maintaining redox balance in cancer cells, further suggesting that targeting GLS could be a novel therapeutic strategy for the treatment of human cancers. In certain glioblastoma cells, however, silencing GLS inhibits cell proliferation but fails to eliminate the cancer cells. A detailed study revealed that a compensatory anaplerotic mechanism catalyzed by pyruvate carboxylase is induced to enable the cells to bypass the need for glutamine.[59] Glucose-derived pyruvate provided by pyruvate carboxylase is necessary when GLS is silenced, indicating that targeting both GLS and pyruvate carboxylase could produce synthetic lethality. In addition, two recent studies have identified GLS as a critical player in mediating resistance to therapies in glioblastoma and T-cell acute lymphoblastic leukemia. Tanaka et al.[60] reported that GLS expression and glutamate levels are elevated following mTOR kinase inhibitor treatment in glioblastoma cells and targeting both mTOR kinase and GLS results in synergistic tumor cell death. In T-cell acute lymphoblastic leukemia, Herranz et al. identified glutaminolysis as a crucial pathway for cell growth downstream of NOTCH1. Knockout of GLS led to significant anti-leukemic effects, which is further enhanced by anti-NOTCH1 therapy in NOTCH1-induced T-ALL.[61]

Unlike GLS, the exact role of GLS2 in cancer is still controversial and not completely understood. Early reports have shown that GLS2 is a p53 target gene that is important for the control of cellular ROS level through regulating GSH production.[62,63] Overexpression of GLS2 in cancer cells inhibits tumor growth in vivo, which is consistent with a tumor-suppressing function. In contrast, later studies demonstrated that GLS2 is overexpressed in certain type of cancers and that silencing GLS2 inhibits tumor growth.[64,65] Nevertheless, it is well accepted that like GLS, GLS2 also has an important role in maintaining cellular redox homeostasis. Cancer cells must maintain redox homeostasis to ensure survival in vivo by regulating endogenous and exogenous oxidative stress. Inhibiting GLS2 can disrupt the redox balance in cancer cells and sensitize them to oxidative stress.[66] Moreover, a recent study revealed that glutaminolysis has a crucial role in ferroptosis, an iron-dependent, non-apoptotic cell death.[67] Genetic and pharmacological inhibition studies demonstrated that GLS2 but not GLS is required for ferroptosis. The function and regulation of GLS in cancer is likely context dependent and warrants further investigation.

GLUTAMATE DEHYDROGENASE

The initial deamination of glutamine by GLS yields glutamate and ammonia. Glutamate is then converted to α-KG, a TCA cycle intermediate, to produce ATP and anabolic carbons for the synthesis of amino acids, nucleotides, and lipids. Several enzymes are involved in the conversion of glutamate to α-KG in the mitochondria. These include GDH1, GOT2 and GPT2. Jin et al.[12] recently reported that RNAi-mediated knockdown of GDH1, but not GPT2 or GOT2, results in significantly decreased α-KG production accompanied by reduced glutaminolysis, decreased anabolic glutamine-dependent RNA biosynthesis and elevated ROS levels in breast cancer and lung cancer cells. Previous studies support that the activation of GDH tightly correlates with

increased glutaminolysis, further supporting GDH as a critical regulator of glutamine metabolism.[68] Conversion of glutamate to α-KG by GDH is carried out via oxidative deamination. There are two isozymes of GDH: GDH1 is expressed ubiquitously whereas GDH2 has only been found in the retina, testis and brain.[69] The activity of mammalian GDH, one of the most allosterically regulated enzymes, is subject to complex allosteric regulation that depends on the metabolic status of the cell.[70] It has been shown that palmitoyl CoA, GTP and ATP can inhibit GDH activity, whereas ADP and leucine serve as allosteric activators.[71] In addition to allosteric regulation, GDH is also regulated through a post-translational modification. Previous reports demonstrate that SIRT4, a mitochondrial enzyme, utilizes NAD to ADP-ribosylate and downregulate GDH activity in pancreatic β-cells.[72] Loss of SIRT4 activates GDH and therefore promotes amino acid-stimulated insulin secretion.[72] The complexity of regulation of GDH in cells places this enzyme in a unique metabolic regulatory step.

The importance of GDH regulation is underscored by the finding that dysregulation of GDH caused by a dominant mutation leads to hyperinsulinism/hyperammonemia (HI/HA).[73] The function of GDH in cancer has been elusive until recently. Yang et al. reported that GDH1 is required for glioblastoma cells to maintain survival under limited glucose supply. Glucose deprivation activates GDH activity and inhibition of GDH1 sensitizes glioblastoma cells to glucose withdrawal.[68] Similarly, Choo et al. also reported that inhibition of GDH1 leads to cell death in TSC$^{-/-}$ cells under glucose deprivation, while suppression of GOT and GPT shows no effect. Treatment with the mTOR inhibitor rapamycin prevents glucose deprivation-induced cell death but fails to do so when GDH1 activity was blocked.[74] These studies suggest a pro-survival role of GDH1 in cancer cells under nutrient stress such as low glucose.

Furthermore, recent reports have shown that GDH is critically involved in the regulation of redox homeostasis in many types of cancer.[12,75] Jin et al. showed that GDH1 expression is upregulated in breast and lung cancers. Silencing GDH1 in human cancer cells led to elevated ROS level and decreased cell proliferation both in vitro and in vivo, indicating that cellular redox homeostasis is disrupted by inhibition of GDH1.[12] Fumarate, the subsequent metabolite of α-KG, directly binds and activates GPx1, a ROS scavenging enzyme, in cancer cells. Inhibition of GDH1 by shRNA or R162, a GDH1-specific inhibitor, decreases fumarate levels, which leads to decreased GPx activity and reduced ROS scavenging capabilities in cancer cells.[12] These findings position GDH1 at a critical node of cellular ROS regulation and further highlight the functional link between glutaminolysis and redox homeostasis.

The involvement of GDH2 in cancer metabolism and growth has also been suggested. A recent report showed that GDH2 is important for supporting the growth of tumors driven by isocitrate dehydrogenase 1 (IDH1) mutations.[76] The authors found that somatic mutation of IDH1, the most common initiating event for secondary glioblastoma, exerts growth inhibitory effects that can be abrogated by GDH2 but not GDH1 overexpression.[76] These findings suggest that targeting GDH2 may selectively benefit cancer patients with IDH1 mutations.

ASPARTATE TRANSAMINASE

In addition to GDH, the conversion of glutamate to α-KG can also be carried out through a reversible transamination process, producing α-KG as well as nonessential amino acids such as alanine and aspartate.[19] In a recent report, aspartate transaminases (GOTs) were identified as critical metabolic enzymes in human pancreatic ductal adenocarcinoma (PDAC) for maintaining redox homeostasis. Genetic inhibition of GOT1 and GOT2 led to elevated ROS levels and suppression of tumor growth in vivo.[34] In another study, Yang et al. found that GOT2 activity is regulated

through acetylation. Acetylation of GOT2 supports ATP production and stimulates production of NADPH to suppress ROS generation.[77] Collectively, these studies have identified GOTs as an important player in redox regulation in human pancreatic cancer.

THERAPEUTIC OPPORTUNITIES

The interest in finding small molecules that target glutamine metabolism started as early as the 1950s, when several glutamine analogs including acivicin, 6-diazo-5-oxo-L-norleucine (L-DON) and azaserine were tested for their anti-tumor activity in both preclinical and clinical studies.[78] All three analogs showed certain degrees of anti-proliferation effects in vitro as well as in xenograft animal models. These analogs non-specifically target several glutaminolytic enzymes such as GLS and therefore inhibit glutamine-dependent nucleotide biosynthesis. Although gluta- mine analogs introduced the possibility of therapeutically exploiting glutamine addiction for cancer treatment, dose- limiting toxicities including neurotoxicity, gastrointestinal toxicity

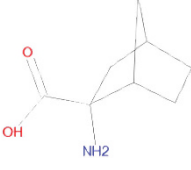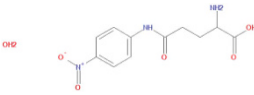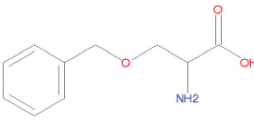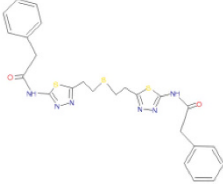**Table 1.** Examples of potential glutaminolytic targets for cancer therapy
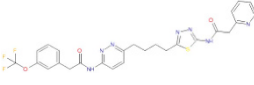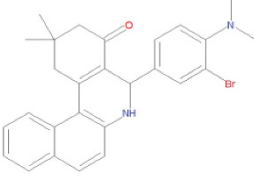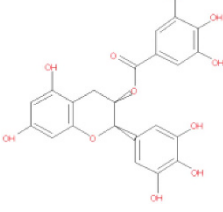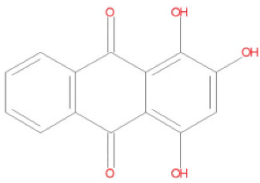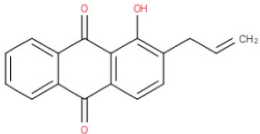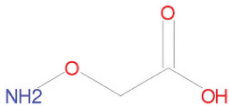
Targets	Inhibitors	Structures	Observations	Ref.
LAT1	BCH		BCH inhibits glutamine/leucine exchange and mTOR activation.	30
ASCT2	GPNA		GPNA inhibits glutamine uptake and induces ROS accumulation by binding to ASCT2. It suppresses lung cancer cell growth in vitro and in vivo.	42, 43
	Benzylserine		Benzylserine inhibits glutamine transport by targeting both LAT1 and ASCT2. It inhibits mTOR signaling and suppresses melanoma cell growth.	44
GLS	BPTES		BPTES and CB-839 specifically inhibit glutaminase and suppress tumor growth in several preclinical animal models including hepatocellular carcinoma, renal cell carcinomas and lymphoma.	57, 58, 61, 81, 83–86
	CB-839			
	Compound 968		Compound 968 suppresses oncogenic transformation induced by Rho GTPases in fibroblasts and shows synergistic anti-tumor activity in combination with mTOR inhibitor in glioblastoma.	53, 60, 87
GDH	EGCG		EGCG/ECG inhibit GDH and modulate insulin secretion.	52, 71, 74

Table 1. (Continued)

Targets	Inhibitors	Structures	Observations	Ref.
	Purpurin		Genetic or pharmacological inhibition of GDH1 by shRNA and R162 attenuates tumor growth *in vitro* and *in vivo*, GDH1 contributes to redox homeostasis in part by regulating GPx activity in cancer cells.	12
	R162			
GOT	AOA		AOA inhibits transaminases such as GOT and GPT, and inhibits breast cancer growth *in vivo*.	89, 90

Abbreviations: AOA, aminooxyacetate; ASCT2, ASC amino acid transporter 2; BCH, 2- aminobicyclo(2,2,1)-heptane-2-carboxylic acid; BPTES, bis-2-[5-phenylacetamido-1, 2, 4-thiadiazol-2-yl] ethyl sulfide; EGCG, epigallocatechin gallate; GDH, glutamate dehydrogenase; GLS, glutaminase; GOT, glutamate oxaloacetate transaminases; GPNA, γ-L-glutamyl-p-nitroanilide; GPT, glutamate pyruvate transaminases; LAT1, L-type amino acid transporter 1; ROS, reactive oxygen species.

and myelosuppression prevent the further development of these glutamine analogs in the clinic.[78]

As a greater appreciation of the role of glutamine in cancer metabolism has emerged in recent years, new efforts have been made to characterize compounds that inhibit specific steps of glutaminolysis, especially those with oncogene-associated glutamine dependency (Table 1).[79–81] For example, SLC1A5 has been identified as the primary glutamine transporter in certain types of cancers and is indispensable for cancer cell proliferation. Therefore, specific inhibition of the transporter to block glutamine uptake could be a promising therapeutic approach for cancer treatment. Indeed, benzylserine and L-γ-glutamyl-*p*-nitroanilide have been shown to effectively inhibit SLC1A5 and suppress tumor growth in lung cancer and melanoma.[43,44] In addition, it has been shown that 2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BCH), an inhibitor of SLC7A5, inhibits mTOR signaling.[30]

GLS is perhaps so far the most extensively studied drug target in the glutaminolysis pathway because of its critical role in glutamine metabolism. Therefore, characterization of new specific inhibitors for GLS has recently become a field of intense research and a variety of small molecule inhibitors have been reported.[57,82] The best characterized GLS inhibitor is bis-2-[5-phenylacetamido-1, 2, 4-thiadiazol-2-yl] ethyl sulfide (BPTES). BPTES allosterically inhibits the dimer-to-tetramer transition of GLS,[54,57] which is critical for activation of the enzyme. Crystal structure analysis of GLS-BPTES showed that BPTES binds to the dimer interface of GLS and consequently stabilizes the inactive tetrameric form of the enzyme.[54] In addition, a number of derivatives of BPTES have been developed,[83] such as CB-839 which is currently in Phase I clinical trials.[58,81] BPTES effectively inhibits the growth of several types of tumors, including Myc-dependent hepatocellular carcinoma,[46] renal cell carcinomas[84] and lymphoma.[49] Consistent with the critical role of glutaminolysis in cellular energetic and redox homeostasis, inhibition of GLS by BPTES decreases ATP levels and profoundly increases ROS levels.[49] In addition, the combination of BPTES and heat shock protein 90 inhibitors has been shown to selectively induce cell death in mTORC1-driven tumor cells.[85]

Interestingly, the expression of pyruvate carboxylase negatively correlates with BPTES sensitivity in lung cancer cell lines, indicating that the sensitivity to GLS inhibition could be affected by the mode of carbon entry into the TCA.[86] Compound 968 (benzophenanthridinone 968) represents another class of GLS inhibitor that has drawn great attention recently.[53,87] Unlike BPTES, 968 is less effective at inhibiting GLS enzyme activity when added to GLS activated by pretreatment with inorganic phosphate, an allosteric activator.[88] Indeed, further study showed that unlike BPTES, 968 does not stabilize an inactive tetrameric state of GLS but rather preferentially binds to an inactive, monomeric state of GLS and inhibits the active conformational changes of the enzyme.[88] 968 treatment suppressed oncogenic transformation induced by Rho GTPases in fibroblast and breast cancer and showed synergistic anti-tumor activity in combination with mTOR inhibition in glioblastoma.[53,60] Since BPTES and 968 regulate GLS activity through distinct allosteric mechanisms, these compounds could be further examined for combination treatment for enhanced GLS inhibition and tumor suppression.

The possibility of targeting the step that converts glutamate to α-KG has also been explored and several inhibitors have been described. It has been shown that epigallocatechin gallate, a flavonoid from green tea, allosterically inhibits GDH and effectively suppresses tumor growth in many types of cancers that rely on glutamine.[71] However, given the fact that epigallocatechin gallate shows numerous pharmacological activities and potentially has multiple targets in the cell, it is unclear how much the inhibition of GDH by epigallocatechin gallate accounts for its anti-tumor activity *in vivo*. Jin et al.[12] recently identified purpurin and its cell permeable derivative R162 as novel GDH inhibitors. Purpurin directly binds and specifically inhibits GDH1 activity *in vitro*. More importantly, purpurin shows no effect on the activity of other NADPH-dependent enzymes such as 6-phosphogluconate dehydrogenase and fumarate hydratase, whereas epigallocatechin gallate dramatically affects the activity of both enzymes. Inhibition of GDH by R162 in cancer cells induces elevated ROS level and inhibits tumor growth *in vivo*, further supporting a critical role of

glutaminolysis in maintaining redox balance in cancer cells and indicating GDH1 as a promising anticancer target.[12]

In addition to GDH, glutamate-dependent transaminases have also been considered as drug targets for modulating glutaminolysis. Aminooxyacetate, which non-specifically inhibits transaminases, has been shown to be effective in inhibiting cell proliferation and tumor growth in several preclinical studies.[89,90]

CONCLUDING REMARKS

Glutamine metabolism in cancer has drawn a substantial amount of interest over the past decade. It has become increasingly clear that glutaminolysis is involved in diverse aspects of cellular functions in cancer cells, many of which are beyond the metabolic role of glutamine. Given the complex interplay between oncogenic signaling and metabolic rewiring, additional metabolic and non-metabolic functions of glutaminolysis in cancer cells will be uncovered. Elucidating how the cancer cells coordinate these functions and reprogram their metabolic pathways in response to environmental stress is of great importance, as these new findings could provide opportunities for therapeutic intervention. Challenges, however, still remain. Because other fast-proliferating cells, such as immune cells, often share similar metabolic pathways with cancer cells,[91] more specific pharmacological interventions that target the particular vulnerabilities of cancer cells will be the future focus of the field.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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