

Glioblastoma Cells Require Glutamate Dehydrogenase to Survive Impairments of Glucose Metabolism or Akt Signaling

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Abstract

Oncogenes influence nutrient metabolism and nutrient dependence. The oncogene *c-Myc* stimulates glutamine metabolism and renders cells dependent on glutamine to sustain viability (“glutamine addiction”), suggesting that treatments targeting glutamine metabolism might selectively kill *c-Myc*-transformed tumor cells. However, many current or proposed cancer therapies interfere with the metabolism of glucose, not glutamine. Here, we studied how *c-Myc*-transformed cells maintained viability when glucose metabolism was impaired. In SF188 glioblastoma cells, glucose deprivation did not affect net glutamine utilization but elicited a switch in the pathways used to deliver glutamine carbon to the tricarboxylic acid cycle, with a large increase in the activity of glutamate dehydrogenase (GDH). The effect on GDH resulted from the loss of glycolysis because it could be mimicked with the glycolytic inhibitor 2-deoxyglucose and reversed with a pyruvate analogue. Furthermore, inhibition of Akt signaling, which facilitates glycolysis, increased GDH activity whereas overexpression of Akt suppressed it, suggesting that Akt indirectly regulates GDH through its effects on glucose metabolism. Suppression of GDH activity with RNA interference or an inhibitor showed that the enzyme is dispensable in cells able to metabolize glucose but is required for cells to survive impairments of glycolysis brought about by glucose deprivation, 2-deoxyglucose, or Akt inhibition. Thus, inhibition of GDH converted these glutamine-addicted cells to glucose-addicted cells. The findings emphasize the integration of glucose metabolism, glutamine metabolism, and oncogenic signaling in glioblastoma cells and suggest that exploiting compensatory pathways of glutamine metabolism can improve the efficacy of cancer treatments that impair glucose utilization. [Cancer Res 2009;69(20):7986–93]

Introduction

Tumor cells display enhanced, autonomous, or otherwise unusual metabolic activities compared with differentiated cells (1). Since Warburg's experiments in the 1920s, the metabolic idiosyncrasies of tumors have been proposed to hold therapeutic opportunities in cancer, and many studies have focused on identifying pathways supporting tumor cell survival and growth.

Thus far, enhanced rates of glucose and glutamine utilization are best established as hallmarks of tumor metabolism (2–4). The central roles of these nutrients derive from their abundance *in vivo*, their use as bioenergetic substrates, and especially from the fact that they provide precursors for the synthesis of lipids, proteins, and nucleic acids. Metabolic flux analysis in proliferating glioblastoma cells showed that the coordinated metabolism of glucose and glutamine enabled a metabolic platform supporting both bioenergetics and biosynthesis (5). These observations suggest that a better understanding of the relationships between glucose and glutamine metabolism will lead to novel approaches to curb tumor growth.

Consistent with the importance of glucose and glutamine for tumor growth, many studies have shown that tumor suppressors and oncogenes influence the metabolism of these nutrients. The phosphatidylinositol 3'-kinase/Akt pathway, which is enhanced in a large fraction of human tumors, positively regulates glucose uptake, glycolysis, and the use of glucose as a macromolecular precursor during cell growth (6, 7). The oncogene *c-Myc* facilitates glutamine metabolism by increasing the expression of surface transporters and enzymes (8, 9). In glioblastoma cells with enhanced *c-Myc* activity, Akt inhibition decreases glycolysis without affecting glutamine consumption, whereas suppression of *c-Myc* reduced the uptake and mitochondrial processing of glutamine (9). Thus, whereas many tumor cells exhibit high rates of glucose and glutamine consumption, distinct oncogenic pathways regulate discrete aspects of the metabolic phenotype.

A surprising consequence of transformation is that it can limit metabolic flexibility *in vitro*. For example, Akt increases not only glucose metabolism but also sensitivity to glucose withdrawal (10). This effect is due to an impaired ability to activate β -oxidation, which normally supports bioenergetics during glucose starvation (10, 11). Similarly, cells with enhanced *c-Myc* activity cannot survive glutamine withdrawal because they cannot maintain pools of tricarboxylic acid (TCA) cycle intermediates in the absence of glutamine (9, 12). These nutrient “addiction” studies suggest that targeting intermediary metabolism will be selectively toxic to cancer cells, with therapeutic specificity defined by the tumor genotype. This might be particularly useful in glucose addiction, because many current cancer therapies alter tumor glucose uptake and are correlated with reduced signal on ¹⁸fluorodeoxyglucose positron emission tomography (¹⁸FDG-PET; ref. 13).

Importantly, however, tumor cells may retain the capacity for metabolic compensation *in vivo* to survive periods of diminished glucose metabolism. Recent studies have documented that a decrease in ¹⁸FDG-PET signal during cancer therapy does not necessarily predict histopathologic resolution or improved patient outcome (14, 15). These studies imply that glycolytic tumors survive stress by activating alternative metabolic pathways and that defining and targeting these pathways will improve cancer

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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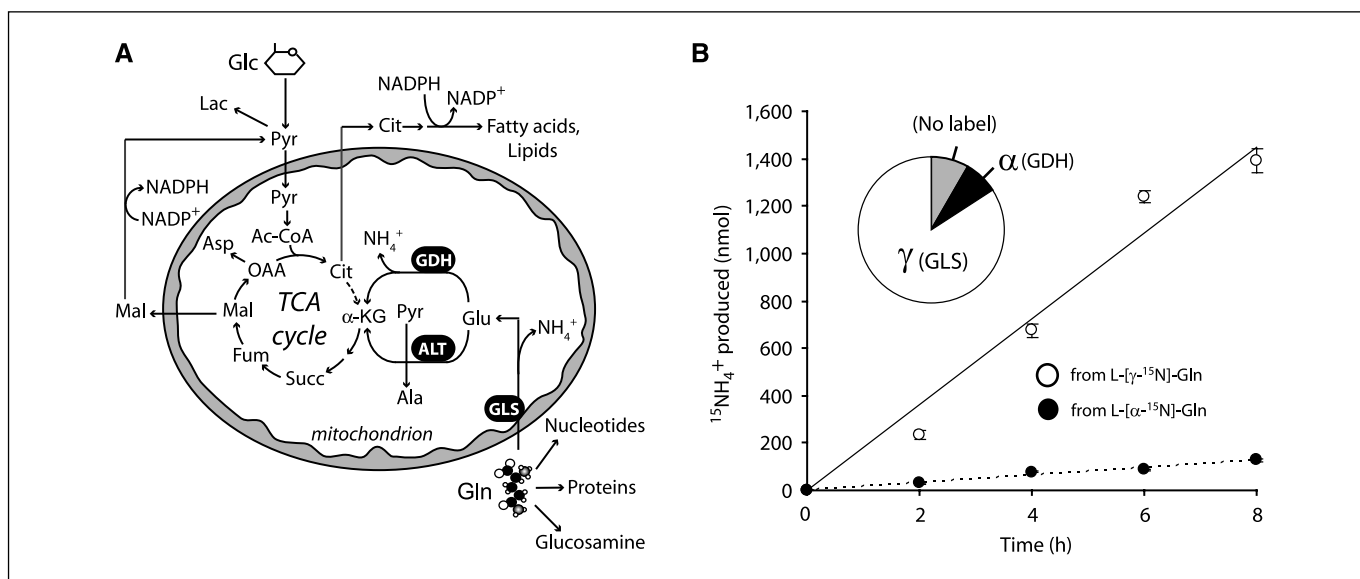


Figure 1. Glutamine metabolism in glioblastoma cells. *A*, glutamine (*Gln*) is a precursor for synthesis of nucleotides, proteins, and glucosamine. It can also be metabolized in the mitochondria, providing carbon to the TCA cycle as α -ketoglutarate (α -KG). Downstream metabolism converts glutamine carbon into OAA and/or acetyl-CoA (*Ac-CoA*), although the latter is predominantly formed from glucose. *B*, glioblastoma cells were cultured in medium supplemented with unlabeled glucose and either L-[α - ^{15}N]glutamine or L-[γ - ^{15}N]glutamine. GLS and GDH activities were followed by measuring transfer of ^{15}N to $^{15}\text{NH}_4^+$. The time course shows the average and SD at each time point for three parallel cultures. The pie graph shows relative contributions of the γ and α nitrogens of glutamine to the total NH_4^+ pool after 8 h. The small amount of unlabeled ammonium (gray wedge) mostly resulted from metabolism of unlabeled glutamate by GDH. *Glc*, glucose; *Pyr*, pyruvate; *Lac*, lactate; *Cit*, citrate; *Glu*, glutamate; *Succ*, succinate; *Fum*, fumarate; *Mal*, malate; *Asp*, aspartate; NH_4^+ , ammonium.

treatment. Given the complementary roles of glucose and glutamine in intermediary metabolism, we studied how glutamine-addicted glioblastoma cells survived glucose deprivation.

Materials and Methods

Reagents. Methyl-pyruvate (CH_3 -Pyr), dimethyl- α -ketoglutarate (dm- α KG), epigallocatechin gallate (EGCG), and 2-deoxyglucose (2-DG) were from Sigma. The isozyme selective (Akt 1/2) inhibitor Akti VIII was from Calbiochem. Isotopes were obtained from Isotec (L-[3- ^{13}C]glutamine) or Cambridge Isotope Laboratories (L-[α - ^{15}N]glutamine; L-[γ - ^{15}N]glutamine).

Cell culture, metabolism, and viability experiments. SF188 cells (UCSF Brain Tumor Registry) were passaged in culture as described (5). These experiments used an SF188-derived cell line overexpressing human Bcl-xL previously used to study metabolism during Akt inhibition (9). The mouse astrocyte cell lines were described previously (16). For all experiments involving nutrient depletion or isotopically enriched nutrients, DMEM was prepared from powder lacking glucose, L-glutamine, phenol red, sodium pyruvate, and sodium bicarbonate (Sigma). This was supplemented with 10% dialyzed fetal calf serum (Hyclone), sodium bicarbonate (42.5 mmol/L), HEPES (25 mmol/L), penicillin/streptomycin (10 units and 10 $\mu\text{g}/\text{mL}$, respectively), and glucose/glutamine as indicated. To measure metabolic rates, 1 million cells were plated into 6-cm dishes and cultured until 90% confluent. At time 0, the cells were rinsed in PBS, fed with 2 mL of the test medium, and cultured. End-point experiments proceeded for 6 to 8 h, then the medium was collected and analyzed for metabolite abundance. Rates were based on the change in metabolite abundance from the start medium. Ammonia was measured with a spectrophotometric assay (Megazyme). Glucose, lactate, glutamine, and glutamate were measured with a chemical analyzer (NOVA Biomedical), and other amino acids were measured by high performance liquid chromatography. For viability measurements, the culture medium and trypsinized cells were combined, stained with trypan blue, and counted with a hemacytometer. Phase contrast images were obtained with a Nikon Eclipse TE300 microscope and processed with MetaMorph (Universal Imaging).

RNA interference. Small interfering RNAs (siRNA) were obtained from Ambion, Inc., reconstituted in water to 20 $\mu\text{mol}/\text{L}$, and transfected using

Effectene (Qiagen). Manufacturer protocols were used to transfect 200 pmol of each siRNA into 1 million cells at a final siRNA concentration of 40 nmol/L. Glucose withdrawal experiments were performed on the 7th day after transfection.

Western blotting. Whole-cell lysates were prepared in radioimmunoprecipitation assay buffer and quantified using the BCA Protein Assay (Thermo Scientific). Protein was separated by SDS-PAGE, transferred to nitrocellulose, and probed with monoclonal antibodies against GDH (Novus Biologicals) or β -actin (Sigma). Detection was performed using SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) and a UVP BioImaging System.

^{13}C nuclear magnetic resonance. Cells were cultured in three 15-cm dishes until 90% confluent, then rinsed and overlaid with 25 mL of medium containing 2 mmol/L L-[3- ^{13}C]glutamine, with or without 10 mmol/L unlabeled glucose. After 7 h, the medium was removed and the cells were rinsed in PBS. The dishes were placed at -80°C for 10 min to halt metabolic activity. Frozen cells were homogenized by sonication in 4 mL of 6% ice-cold perchloric acid. After centrifuging to remove debris, the acid-soluble material was neutralized with 8 N potassium hydroxide and centrifuged. The supernatant was lyophilized, reconstituted in D_2O , and analyzed in a 5-mm nuclear magnetic resonance (NMR) tube. NMR analysis was performed on a Bruker Avance 400 wide bore 9.4T instrument equipped with a 5-mm $^1\text{H}/^{13}\text{C}$ dual probe. ^{13}C spectra were recorded under approximately fully relaxed conditions and broadband proton decoupling. Carbon spectra were acquired under the following conditions: pulse flip angle 45° , repetition time 1.5 s, spectral width 25 kHz, number of data points 64,000, and number of scans $\sim 25,000$. Free induction decays were zero-filled to 131,072 points and apodized with exponential multiplication. Spectra were analyzed using NMR Utility Transform Software (Acorn NMR) and the relative abundances of glutamate C4 and C5 signals were determined by normalizing areas to natural abundance HEPES resonances.

Gas chromatography/mass spectrometry. Enrichment in NH_4^+ was determined using a modification of a published method (17). Briefly, NH_4^+ from 50 to 100 μL of culture medium was converted to aminobutyrate in the presence of oxobutyrate, purified glutamate dehydrogenase (GDH), and NADH (Sigma) for 30 min at 37°C . The sample was applied to an ion exchange column (AG 50W-X4, Bio-Rad), washed with water, and eluted

with 2 mL of 4 N ammonium hydroxide. To determine enrichment in alanine, the culture medium was subjected to the same column purification. Standards containing known ratios of unlabeled/¹⁵N-labeled NH₄⁺ and alanine were prepared using the same method. Samples were evaporated and derivatized in 100 μL of Tri-Sil (Thermo Scientific), then analyzed

by gas chromatography/mass spectrometry (GC/MS) with an Agilent 6890N GC coupled to an Agilent 5973 Mass Selective Detector. The oven temperature was ramped from 70°C to 150°C by 5°C/min, then by 10°C/min to 325°C. The following ion ratios were measured: alanine *m/z* 116 (unenriched) and 117 (enriched) and aminobutyrate *m/z* 130 (unenriched) and 131 (enriched). The atom percent excess of each product was calculated by comparing the ion ratio to the standard curve.

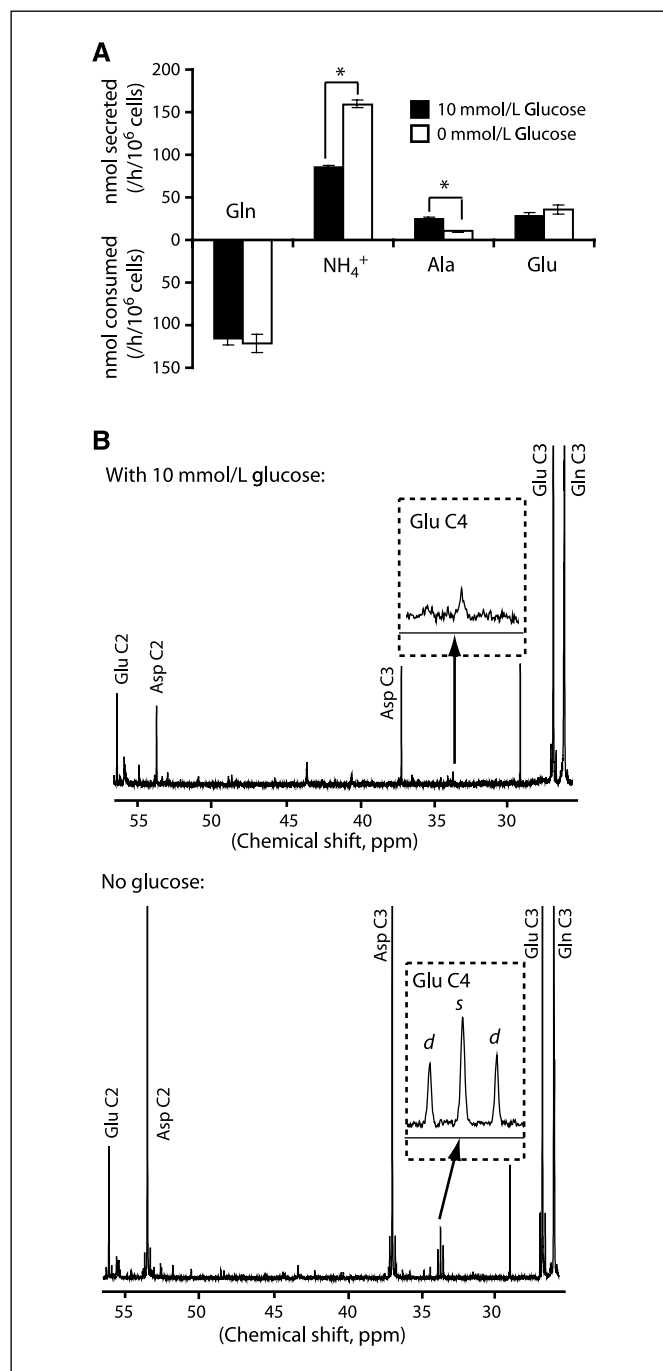


Figure 2. Glucose withdrawal increases mitochondrial glutamine metabolism. **A**, glutamine-dependent metabolic rates in the presence and absence of glucose. Averages and SDs of three independent cultures are shown. *, $P < 0.005$. **B**, ¹³C NMR spectra of metabolites from cells cultured in medium containing L-[3-¹³C]glutamine plus or minus unlabeled glucose. *Inset*, an enlargement of the glutamate C4 resonance at 34 ppm. The doublet (*d*) corresponds to glutamate labeled both at C4 (from acetyl-CoA) and at C3 (from glutamine-derived OAA), and the singlet (*s*) corresponds to glutamate molecules labeled at C4 but not C3. The experiment was performed twice for each condition. *Ala*, alanine.

Results

Glucose withdrawal stimulates mitochondrial glutamine metabolism. We previously determined that SF188 glioblastoma cells metabolized carbon from glutamine in the TCA cycle and secreted ammonia in the process (5). To understand how the cells converted glutamine into the TCA cycle intermediate α -ketoglutarate (α -KG), we compared the activities of glutaminase (GLS) and GDH. These two enzymes generate ammonia from the γ and α nitrogens of glutamine, respectively, and their sequential activity produces α -KG (Fig. 1A). We cultured cells with glutamine that was labeled with ¹⁵N in either the γ - or α -position and followed production of ¹⁵NH₄⁺ by GC/MS (Fig. 1B). After 8 hours, more than 90% of the ammonia in the medium was derived from these two nitrogens (Fig. 1B). More than half ($58 \pm 1\%$) of the glutamine consumed by the cells was metabolized by GLS, and thus this enzyme outpaced all other glutamine-consuming systems combined. Furthermore, the GDH flux was approximately 10% of the GLS flux, consistent with the known contribution of other glutamate-consuming enzymes [e.g., alanine aminotransferase (ALT), aspartate aminotransferase] under glucose-replete conditions (5).

Because these cells normally use carbon from both glucose and glutamine to operate the TCA cycle (Fig. 1A), we tested whether glucose availability influenced glutamine metabolism. Cells were cultured in 10 or 0 mmol/L glucose, and the medium was analyzed to measure the consumption of glutamine and the secretion of by-products of glutamine catabolism (ammonia, alanine and glutamate). Under both conditions, the cells consumed glutamine at a rate at least 10-fold higher than any other amino acid, and alanine and glutamate were by far the most rapidly secreted amino acids (Supplementary Table S1). ATP levels were maintained over the time course (Supplementary Fig. S1). Surprisingly, glucose deprivation did not affect the net utilization of glutamine or of any other amino acid, suggesting that the cells did not enhance amino acid consumption rates to compensate for the loss of glucose (Supplementary Table S1; Fig. 2A). However, glucose deprivation doubled total ammoniogenesis (Fig. 2A), implying an increased allocation of glutamine into ammonia-generating mitochondrial reactions.

Mitochondrial metabolism of glutamine follows two divergent pathways (Fig. 1A). In one, glutamine carbon is used to produce oxaloacetate (OAA) in the TCA cycle. In the other, glutamine-derived malate leaves the mitochondria to be used as a minor source of pyruvate and acetyl-CoA, both of which are predominantly formed from glucose carbon (5). To determine how glucose withdrawal affected these pathways, we cultured cells in L-[3-¹³C]glutamine in the presence or absence of 10 mmol/L glucose and analyzed intracellular metabolites by ¹³C NMR. In the presence of glucose, glutamine enriched the OAA pool as shown by the labeling in aspartate carbons 2 and 3 (Fig. 2B, top). Glutamine was only a minor source of acetyl-CoA, as shown by the minimal labeling in glutamate carbon 4, which arises from acetyl-CoA (Fig. 2B, top). When glucose was withdrawn, glutamine not only

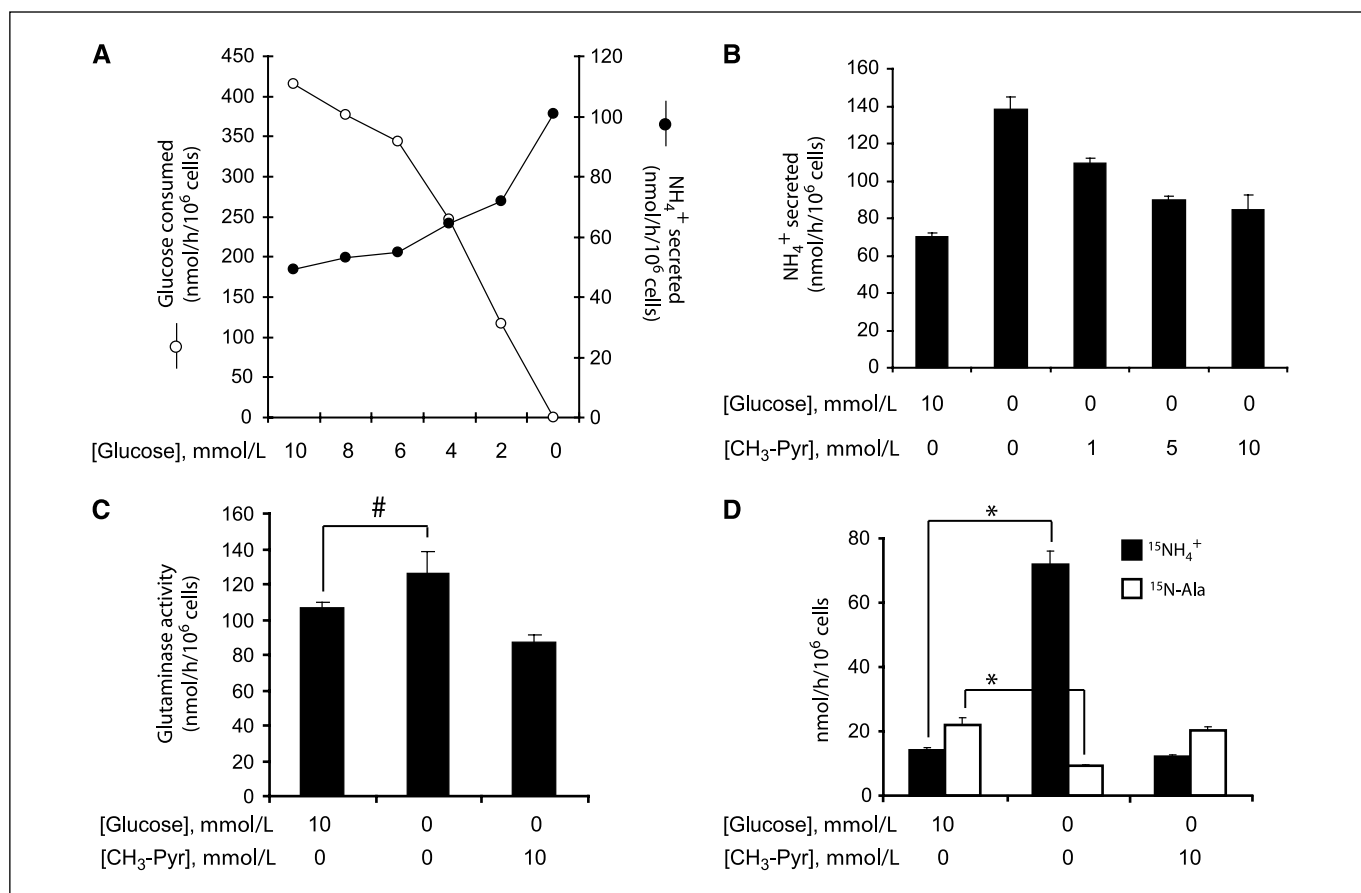


Figure 3. Glucose withdrawal activates GDH. *A*, cells were cultured in decreasing glucose concentrations, and the rates of glucose consumption and ammonia production were measured. *B*, total ammoniogenesis was measured in cells cultured with or without glucose and with increasing concentrations of methyl-pyruvate (CH₃-Pyr). The average and SD of three independent cultures are shown. *C*, GLS activity was measured by following transfer of ¹⁵N from L-[γ-¹⁵N]glutamine to ¹⁵NH₄⁺ in the presence or absence of glucose and CH₃-Pyr. The average and SD of three independent cultures are shown. #, *P* < 0.05. *D*, cells were cultured in L-[α-¹⁵N]glutamine and the accumulation of ¹⁵NH₄⁺ (GDH activity) and of ¹⁵N-alanine (ALT activity) was measured in the presence or absence of glucose and CH₃-Pyr. The average and SD of three independent cultures are shown. *, *P* < 0.0005.

enriched the OAA pool but also contributed carbon to pyruvate/acetyl-CoA, because the labeling in glutamate carbon 4 (C4) increased 5-fold (Fig. 2*B*, bottom). Labeling in glutamate C5, also derived from acetyl-CoA, was similarly increased (data not shown). To confirm increased enrichment of the pyruvate pool, we measured ¹³C content in secreted lactate. In the presence of glucose, less than 5% of the lactate was derived from glutamine, but this percentage gradually increased as the glucose concentration in the medium declined (Supplementary Fig. S2*A*). However, the total abundance of ¹³C-labeled lactate fell during glucose deprivation (Supplementary Fig. S2*B*), as would be expected if more of the glutamine-derived pyruvate was converted to acetyl-CoA and oxidized in the TCA cycle. Thus, these cells use glutamine to produce OAA, pyruvate, and acetyl-CoA and to maintain the TCA cycle function in the absence of glucose.

Glucose withdrawal stimulates GDH activity. We next studied the mechanism by which glucose deprivation increased total ammoniogenesis. Glucose withdrawal caused a gradual increase in ammonia production (Fig. 3*A*). This could be reversed with CH₃-Pyr (Fig. 3*B*), a pyruvate analogue metabolized in the mitochondria (18), suggesting that the increased ammonia production resulted from reduced delivery of pyruvate to the mitochondria. Surprisingly, glucose deprivation only marginally increased GLS activity (Fig. 3*C*). Instead, the excess ammonia was due to a large increase

in GDH activity, which was completely reversed by CH₃-Pyr (Fig. 3*D*). The opposite trend was observed for alanine secretion (Fig. 3*D*). Thus, glucose withdrawal caused a 12-fold increase in the ratio of GDH to ALT activity. This effect was not specific for glioblastoma cells because withdrawal of glucose from mouse embryonic fibroblasts also increased ammonia production and GDH activity (Supplementary Fig. S3).

Loss of GDH activity limits the use of glutamine carbon in the TCA cycle and sensitizes glioblastoma cells to glucose withdrawal. The abrupt increase in GDH activity during glucose withdrawal raised the possibility that GDH compensates for glucose deprivation and allows cells to maintain viability. To test this, we used siRNAs targeting the two GDH isoforms, encoded by the genes *GLUD1* and *GLUD2*. An siRNA directed against *GLUD2* did not affect GDH abundance (data not shown), whereas two siRNAs directed against the *GLUD1* transcript reduced GDH protein abundance and enzyme activity (Fig. 4*A*). Cells containing either of these siRNAs were indistinguishable from cells transfected with a control siRNA when glucose was present, but they lost viability in the absence of glucose for 24 hours (Fig. 4*B* and *C*). CH₃-Pyr completely rescued the viability of the glucose-deprived cells, as did dm-αKG, an analogue of α-KG (Fig. 4*B* and *C*), which like CH₃-Pyr suppressed GDH activity (Supplementary Fig. S4). To determine the effect of GDH knockdown on the TCA cycle, we

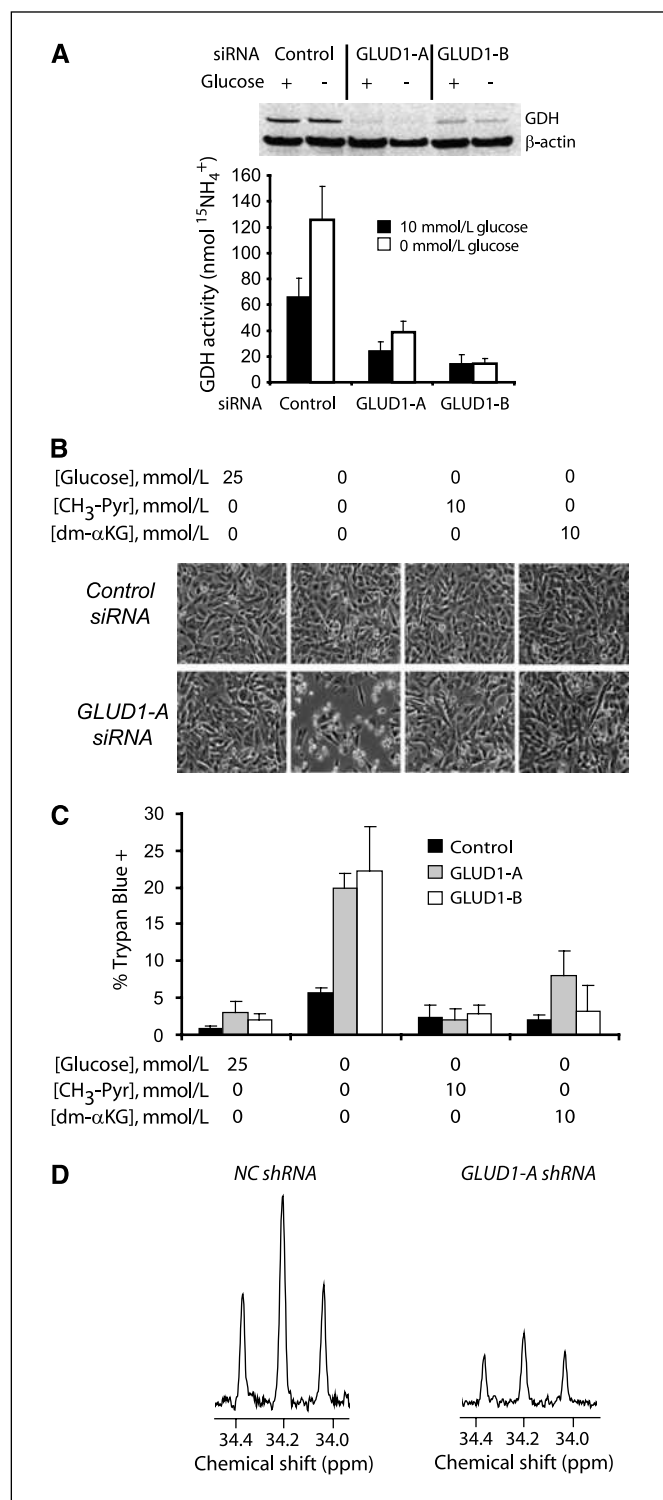


Figure 4. Glioblastoma cells require GDH to survive glucose deprivation. *A*, cells were transfected with a control siRNA or siRNAs directed against the *GLUD1* transcript. The effect on GDH protein abundance (*top*) and flux (*bottom*) was determined in the presence and absence of glucose. The average and SD of three independent cultures are shown. *B* and *C*, cells transfected with the control or *GLUD1* siRNAs were subjected to glucose withdrawal, and the effects on morphology and viability were determined. The metabolites CH₃-Pyr and dm- α KG were tested for their ability to rescue viability in glucose-deprived cells. The average and SD of three independent cultures are shown for each condition. *D*, glucose-deprived cells expressing a control (NC) shRNA or a shRNA against the *GLUD1* transcript (*GLUD1-A*) were cultured with L-[3- ^{13}C]glutamine. Labeling in carbon 4 of glutamate was analyzed by ^{13}C NMR.

generated a cell line with stable short hairpin RNA (shRNA)-mediated suppression of GDH (Supplementary Fig. S5A,B). Compared with cells expressing a control shRNA, GDH knockdown cells lost viability in the absence of glucose unless supplemented with CH₃-Pyr (Supplementary Fig. S5C). We cultured these cells in L-[3- ^{13}C]glutamine and analyzed intracellular metabolites with ^{13}C NMR. Suppression of GDH activity impaired the conversion of glutamine to acetyl-CoA, as shown by decreased labeling in glutamate C4 (Fig. 4D). This effect was not due to global metabolic defects because labeling in aspartate was similar between the two cell lines (Supplementary Fig. S5D).

Inhibition of glycolysis or Akt signaling stimulates GDH activity. We next tested whether inhibitors of glycolysis could influence GDH activity. First, we treated cells with 2-DG, a competitive hexokinase inhibitor. Addition of 2-DG suppressed lactate production and increased ammoniogenesis (Fig. 5A). The drug also increased GDH activity, and this effect was reversed by CH₃-Pyr (Fig. 5B). Next, we treated cells with an Akt inhibitor previously reported to reduce glycolysis in these cells (9). Similar to the effects of glucose withdrawal, the Akt inhibitor suppressed alanine secretion and mildly increased GLS activity (data not shown), whereas GDH activity was significantly enhanced (Fig. 5C). Addition of CH₃-Pyr suppressed GDH activity during treatment with the Akt inhibitor (Fig. 5C). Furthermore, mouse astrocytes expressing a constitutively active Akt allele displayed chronically suppressed GDH activity compared with parental cells (Fig. 5D). Together, these data show that pharmacologic impairment of glycolysis enhances GDH activity, and that Akt suppresses GDH activity through its effects on glucose metabolism.

A GDH inhibitor sensitizes glioblastoma cells to drugs that suppress glycolysis. We next tested whether we could recapitulate the effects of GDH knockdown using EGCG, an abundant green tea polyphenol that inhibits GDH *in vitro* (19). EGCG blunted maximal GDH activity in glucose-deprived cells (Fig. 6A). Like the effect of GDH knockdown, EGCG enhanced cell death in the absence of glucose, and addition of either CH₃-Pyr or dm- α KG rescued viability (Fig. 6B). Finally, to test whether EGCG could enhance the effect of drugs that limit glucose metabolism, we cultured cells in the presence of glucose with EGCG and either the Akt inhibitor or 2-DG. EGCG increased the effect of both these inhibitors on cell death (Fig. 6C).

Discussion

This decade has seen renewed interest in understanding the metabolic activities that support tumor cell survival and growth. The abundance of data showing that mutations in oncogenes and tumor suppressors influence glucose metabolism, coupled with the utility of ^{18}F FDG-PET, have raised the expectation that metabolic therapies directed against glycolysis will be effective in cancer. However, recent studies showing imperfect correlation between improvement on ^{18}F FDG-PET and patient survival have tempered this hope. Such studies suggest that tumors are capable of surviving periods of reduced glucose metabolism by using alternative metabolic pathways. Finding and targeting these survival pathways may ultimately improve the long-term efficacy of treatments that interfere with tumor glucose metabolism. Here, we identified such a survival pathway catalyzed by GDH in a highly glycolytic glioblastoma cell line.

The emerging picture of tumor cell metabolism is that a complex network links signal transduction with the metabolism of glucose,

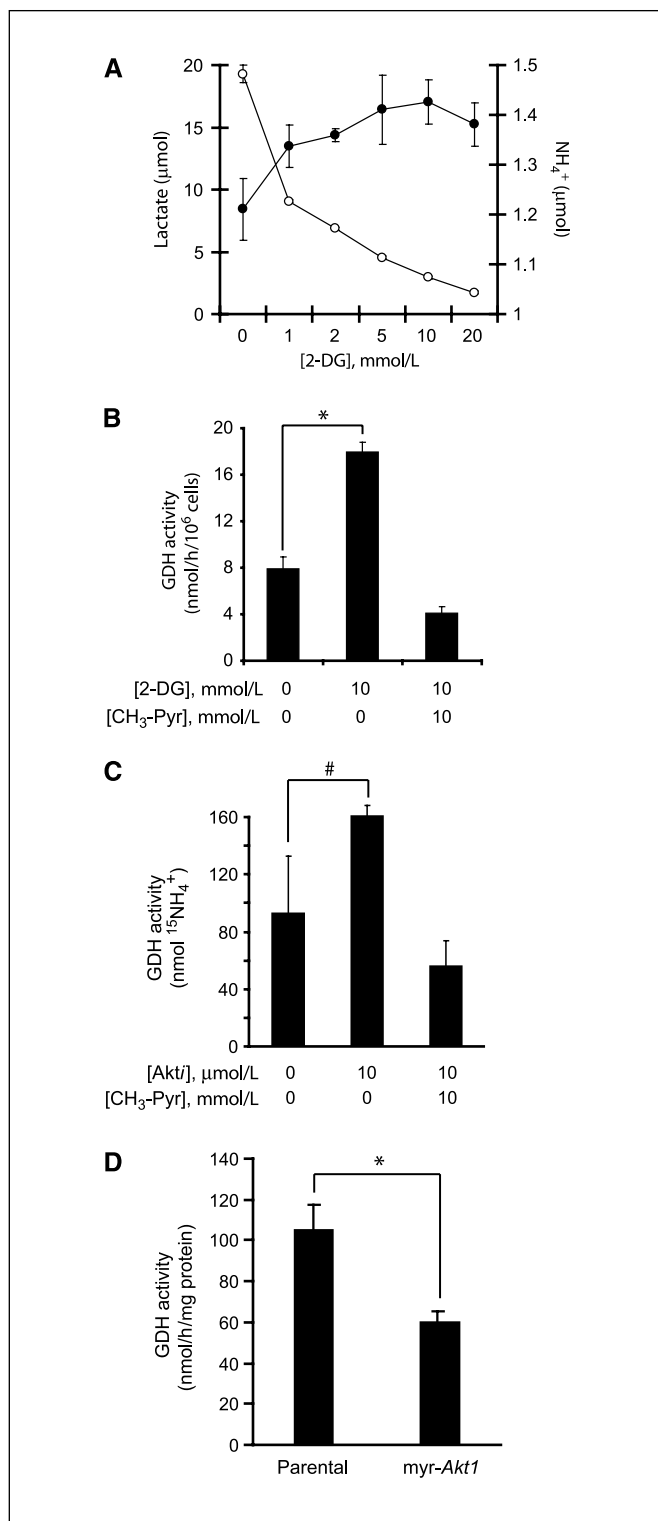


Figure 5. Inhibition of glycolysis or Akt signaling activates GDH. *A*, glioblastoma cells were cultured in increasing concentrations of 2-DG and the release of lactate (○) and NH₄⁺ (●) was measured. *B*, cells were cultured in 4 mmol/L L-[α-¹⁵N]glutamine and the accumulation of ¹⁵NH₄⁺ was measured in the presence or absence of 2-DG and CH₃-Pyr. *C*, cells were cultured with an Akt inhibitor previously shown to suppress glycolysis in these cells (9). The effect on GDH activity in the absence and presence of CH₃-Pyr was determined. *D*, GDH activity was compared between immortalized mouse astrocytes lacking or containing a constitutively active Akt allele (*myr-Akt1*). In all experiments, the average and SD of three independent cultures are shown for each condition. #, $P < 0.05$; *, $P < 0.005$.

glutamine, and other nutrients. Understanding this network will enhance efforts to develop metabolically targeted cancer therapies. This work emphasizes how distinct signaling pathways can influence glucose and glutamine metabolism in the same cells. In the glioblastoma cells we studied, Akt facilitates glucose metabolism but exerts no effect on total glutamine consumption (9). Rather, c-Myc stimulates glutamine metabolism by increasing the expression of amino acid transporters and GLS, resulting in increased glutamine catabolism and increased sensitivity to glutamine withdrawal (8, 9, 12). c-Myc activates a metabolic program in which glucose-replete cells require transamination reactions to produce α-KG (9). Thus, whereas Akt directs the delivery of glucose-derived acetyl-CoA to the TCA cycle, c-Myc

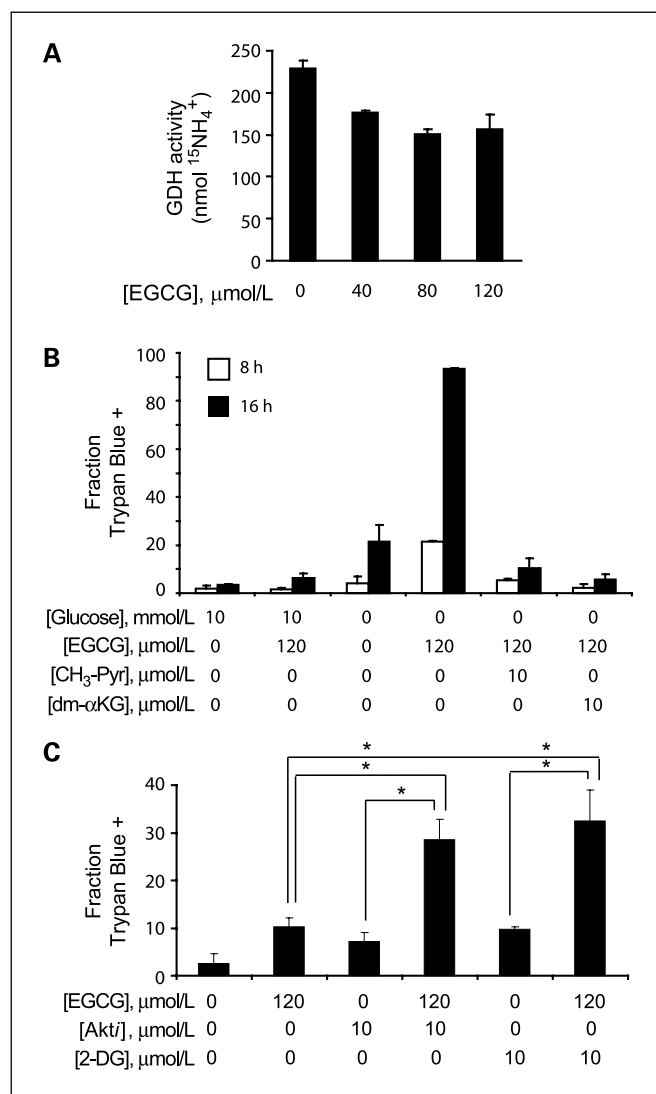


Figure 6. A GDH inhibitor sensitizes glioblastoma cells to glucose withdrawal and to inhibitors of glycolysis and Akt signaling. *A*, the effect of EGCG on GDH activity was determined in glucose-deprived cells. *B*, glioblastoma cells were cultured in the presence or absence of glucose, EGCG, CH₃-Pyr, and dm-αKG. The effects of these treatments, alone or in combination, were determined on cell viability at 8 and 16 h. *C*, glioblastoma cells were cultured in the presence or absence of EGCG, an Akt inhibitor, or 2-DG, alone or in combination. After 24 h, viability was determined by trypan blue staining. In all experiments, the average and SD of three independent cultures are shown for each condition. *, $P \leq 0.005$.

directs the delivery of glutamine-derived α -KG and ultimately OAA for anaplerosis and cell growth. Glucose-replete cells can tolerate suppression of GDH because there are other sources of both α -KG and acetyl-CoA.

The current work adds to this picture by describing an alternative pathway that is activated in glucose-starved cells. The new pathway involves a large increase in GDH activity compared with ALT, which is the most active transaminase when glucose is present (5). Unlike transamination reactions, GDH has the advantage of delivering α -KG to the TCA cycle without the expenditure of a keto-acid, which could otherwise be oxidized to supplement ATP production. For example, the cells we studied displayed aspartate aminotransferase activity during glucose withdrawal, as shown by the transfer of ^{13}C from glutamine to aspartate (Fig. 2B). This reaction converts glutamate to α -KG but consumes OAA in the process. Thus, the pathway serves as a "minicycle" that generates aspartate but does not produce OAA for traditional TCA cycling (20). The concomitant induction of GDH would yield net OAA and therefore support ongoing citrate synthesis and function of the TCA cycle. Moreover, under these conditions, glutamine metabolism also generated acetyl-CoA for the TCA cycle in a GDH-dependent manner. This may explain how the cells maintained their ATP content in the absence of glycolysis (Supplementary Fig. S1).

The effects of glucose withdrawal on both ALT and GDH were reversed by $\text{CH}_3\text{-Pyr}$ (Fig. 3D). Presumably, this molecule stimulates ALT by supplying a large intracellular pyruvate pool for transamination. Because both ALT and GDH consume glutamate, competition between the two enzymes might contribute to the negative effect of $\text{CH}_3\text{-Pyr}$ on GDH. However, the large discrepancy between GDH suppression (~ 60 nmol/h/million cells) and ALT induction (only ~ 10 nmol/h/million cells) suggests the involvement of additional mechanisms.

GDH has not received much attention in cancer cell metabolism, probably because its activity is suppressed during robust glycolysis *in vitro*. GDH is a widely expressed homohexameric enzyme localized to the mitochondrial matrix, where it coordinates carbon and nitrogen metabolism. It determines the rate of oxidative degradation of glutamate and other nonessential amino acids and thus is uniquely poised to respond to glucose deprivation. The regulation of GDH activity is extremely complex and involves allosteric effects, posttranslational modifications, and other levels of control (21, 22). Suppression of GDH activity by glucose is not restricted to glioblastoma because we also observed it in mouse embryonic fibroblasts (Supplementary Fig. S3) and another study observed it in myeloma and hybridoma cells (23). The mechanism for GDH activation during glucose withdrawal is independent of changes in mRNA and protein levels (data not shown and Fig. 4A). Because the forward reaction (oxidative deamination of glutamate) uses NAD^+ as a cofactor, it is possible that the low cellular NAD^+/NADH ratio of highly glycolytic cells holds GDH activity in check. A similar phenomenon has been observed in brain slices where glucose deprivation increased the cytoplasmic and mitochondrial NAD^+/NADH ratio and ammoniogenesis (24). It is significant that in the cells studied here, there was no toxicity associated with the increased production of ammonia when glucose was withdrawn. Rather, the additional GDH activity sustained cell viability. GDH serves a similar compensatory role in plants. Mutant strains of *Arabidopsis* lacking GDH activity grew normally in carbon-replete conditions, but could not survive carbon starvation induced by prolonged

growth in the dark. Viability was restored to the mutants simply by providing an exogenous carbohydrate source (25). Thus, we conclude that derepression of GDH is necessary for some cells to adapt to and survive low-glucose conditions.

It is not known to what extent the metabolism of cultured tumor cells parallels tumor metabolism *in vivo*, but evidence suggests that many core metabolic activities are shared between settings. First, ^{18}F -FDG-PET and ^1H magnetic resonance spectroscopy reveal robust glucose consumption and lactate production in aggressive tumors. Second, expression studies have shown that enzymes involved in glucose and glutamine metabolism are abundantly expressed in tumor tissues and in some cases predict patient outcome (26–29). Third, the inhibition of some of these enzymes impairs tumor growth in animal models (30–33). Fourth, some mechanisms of metabolic compensation, particularly autophagy, allow cells to survive nutrient stress *in vitro* and *in vivo* (34–36). Likewise, GDH inhibition may increase the efficacy of treatments that interfere with glucose metabolism. There are many such treatments already in use that could benefit from concomitant blockade of GDH. Alkylators and other DNA-damaging agents suppress glycolysis *in vitro* and *in vivo* through poly(ADP-ribose) polymerase-dependent depletion of cytoplasmic NAD (37–39). The kinase inhibitors imatinib and rapamycin inhibit glycolysis *in vitro* by reversing the effects of oncogenic signaling pathways on glucose metabolism; they also suppress ^{18}F -FDG-PET signal in some tumors (15, 40–42).

A major challenge in cancer therapeutics is to develop strategies that antagonize tumor cell survival without causing dose-limiting toxicity. Previous efforts to inhibit tumor glutamine metabolism were complicated by nausea, mucositis, and pancytopenia (43). In this regard, tea polyphenols are intriguing because they are consumed in large quantities by millions of people worldwide and because a number of epidemiologic studies have shown the benefits of green tea in preventing the initiation or progression of cancer (44). EGCG in particular has a number of pharmacologic properties that could independently suppress tumor cell growth (45). The relevance of the activity of EGCG as a GDH inhibitor is unknown, although this has been proposed as a mechanism to explain the protective effect of green tea against diabetes (19). In the glioblastoma cells we studied, the effect of EGCG perfectly mimicked GDH knockdown in that it was enhanced during decreased glucose metabolism and reversed by providing GDH-dependent nutrients. Thus, it will be interesting to test whether genetic or pharmacologic impairment of GDH activity can suppress tumor growth or enhance the effect of conventional cancer therapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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