# Hominoid-specific enzyme GLUD2 promotes growth of *IDH1*$^{R132H}$ glioma

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**Somatic mutation of isocitrate dehydrogenase 1 (*IDH1*) is now recognized as the most common initiating event for secondary glioblastoma, a brain tumor type arising with high frequency in the frontal lobe. A puzzling feature of *IDH1* mutation is the selective manifestation of glioma as the only neoplasm frequently associated with early postzygotic occurrence of this genomic alteration. We report here that *IDH1*$^{R132H}$ exhibits a growth-inhibitory effect that is abrogated in the presence of glutamate dehydrogenase 2 (GLUD2), a hominoid-specific enzyme purportedly optimized to facilitate glutamate turnover in human forebrain. Using murine glioma progenitor cells, we demonstrate that *IDH1*$^{R132H}$ exerts a growth-inhibitory effect that is paralleled by deficiency in metabolic flux from glucose and glutamine to lipids. Examining human gliomas, we find that glutamate dehydrogenase 1 (GLUD1) and GLUD2 are overexpressed in *IDH1*-mutant tumors and that orthotopic growth of an *IDH1*-mutant glioma line is inhibited by knockdown of GLUD1/2. Strikingly, introduction of GLUD2 into murine glioma progenitor cells reverses deleterious effects of *IDH1* mutation on metabolic flux and tumor growth. Further, we report that glutamate, a substrate of GLUD2 and a neurotransmitter abundant in mammalian neocortex, can support growth of glioma progenitor cells irrespective of *IDH1* mutation status. These findings suggest that specialization of human neocortex for high glutamate neurotransmitter flux creates a metabolic niche conducive to growth of *IDH1* mutant tumors.**

tumor metabolism | astrocytoma | oligodendroglioma

Malignant transformation is widely recognized to require metabolic reprogramming to enable rapid expansion of biomass (1). Reports that mutation or overexpression of metabolic enzymes can drive oncogenesis have spurred intense investigation into metabolic vulnerabilities that distinguish malignant and normal tissue (2); however, the extent to which specialized metabolism of normal differentiated tissues cooperates with particular oncogenes to facilitate tumor growth has largely escaped notice (3, 4).

Glioblastoma (GBM) is a highly aggressive brain malignancy and, until recently, all oncogenes identified in this tumor type constitute components of growth factor signaling pathways that activate anabolic processes. The discovery that mutation of isocitrate dehydrogenase 1 (*IDH1*), or the homologous gene *IDH2*, is the initiating event for the majority of secondary GBM and lower-grade diffuse glioma (5–7) suggests a novel mechanism for gliomagenesis. Oncogenic mutations altering IDH1 and IDH2 enzymes redirect metabolic flux to generate high concentrations of 2-hydroxyglutarate (2-HG) (8, 9), a metabolite that seems to initiate gliomagenesis via altered activity of α-ketoglutarate–dependent enzymes controlling hypoxia-inducible factor stability, epigenetic marks, and/or collagen maturation (10). Although *IDH1* mutation promotes an undifferentiated phenotype (10, 11), the role of mutant enzyme in tumor growth is not clear (12, 13). Because *IDH1*-WT (*IDH1*$^{WT}$) and *IDH1*-mutant GBMs are distinct diseases (14) and differ categorically in metabolic processes to support proliferation (15), effects of *IDH1* mutation on *IDH1*$^{WT}$ glioma lines must be interpreted with caution. Herein, to gain greater understanding of the mechanisms by which *IDH1*-mutant gliomas support biomass expansion, we sought to identify determinants of growth in glioma progenitor cells bearing *IDH1* mutation.

Because the majority of *IDH1*-mutant GBMs harbor mutations in *p53* and display proneural gene expression signature (16), we chose for study a model system of *p53*-deficient cells competent to generate proneural gliomas at high efficiency (17). Cultured neural stem cells from brains of newborn Nestin-tva *p53* $^{-/-}$ or Nestin-tva *p53* $^{+/-}$ mice formed gliomas with 100% penetrance following infection with replication-competent avian sarcoma leukosis virus long terminal repeat with splice acceptor (RCAS) vector encoding PDGF-B (PDGF/RCAS) and subsequent implantation into forebrain of immunocompromised mice (Fig. 1*A*) and hence are referred to as glioma progenitor cells. Whereas cultures infected with PDGF/RCAS generated gliomas that caused all host mice to become symptomatic within 2–4 wk post-implantation, cultures infected with RCAS vector encoding IDH1$^{R132H}$ (IDH1$^{R132H}$/RCAS) failed to generate expanding grafts for a period of 6 mo following implantation (Fig. 1*A*).

Examining growth of *p53* $^{-/-}$ glioma progenitors in vitro, we observed that both knockdown of IDH1 or expression of IDH1$^{R132H}$ strongly decreased bulk culture growth and clone formation (Fig. 1 *B* and *C*). As expected, supernatants of cultures expressing

## Significance

**Mutation of isocitrate dehydrogenase 1 (*IDH1*) is believed to be the initiating event for the majority of secondary glioblastomas and lower-grade diffuse gliomas; however, the basis for tissue specificity of oncogenesis initiated by *IDH1* mutation has not been apparent. We report evidence to suggest that specialization of human neocortex for glutaminergic neurotransmission provides a metabolic niche particularly suited for growth of *IDH1*$^{R132H}$ glioma. Our findings reveal that *IDH1*-mutant enzyme challenges growth of murine glioma progenitor cells but that these cells thrive if they are engineered to express the hominoid-specific brain enzyme GLUD2, a mitochondrial enzyme that converts glutamate to alpha-ketoglutarate in human cortex. The current findings raise the possibility that evolutionary changes contributing to human cognitive abilities may have conferred vulnerability to brain tumors driven by *IDH1* mutation.**

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A

| Cell line | p53 status | Mice with Gliomas PDGF-B | Mice with Gliomas hIDH1$^{R132H}$ |
|---|---|---|---|
| 1 | p53KO | ND | 0/9 |
| 2 | p53HET | 3/3 | 0/9 |
| 3 | p53HET | 3/3 | 0/9 |
| 4 | p53HET | 3/3 | 0/9 |
| 5 | p53KO | 6/6 | 0/9 |

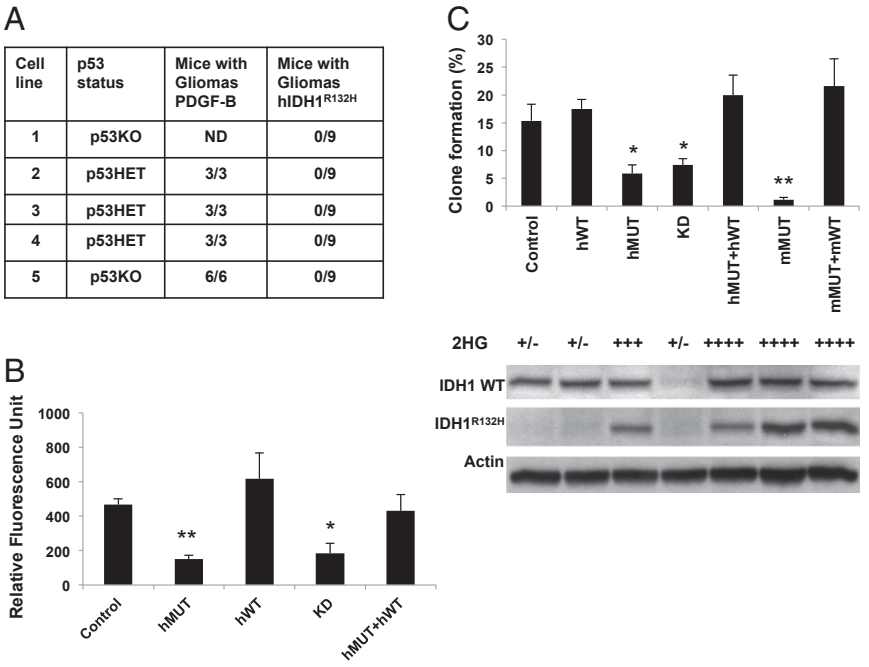


**Fig. 1.** IDH1$^{R132H}$ inhibits growth of murine glioma progenitor cells. (*A*) Incidence of glioma formation with *p53* $^{+/-}$ or *p53* $^{-/-}$ neural stem cultures expressing PDGF-B or human IDH1$^{R132H}$ (hIDH1$^{R132H}$). ND, not determined. (*B*) CyQuant assay of glioma progenitors (line 5 in *A*) expressing either no exogenous protein (control), human IDH1$^{R132H}$ (hMUT), human IDH1$^{WT}$ (hWT), shRNA to murine IDH1 (KD), or hMUT and hWT (hMUT + hWT). For *B* and *C*, bars represent mean ± SEM for three or more determinations; *$P < 0.005$, **$P < 0.0005$ vs. control, *t* test. (*C*) (*Top*) Clone formation assay. mMUT, a clonal line expressing murine IDH1$^{R132H}$; mMUT + mWT, mMUT clonal line rescued with IDH1$^{WT}$/RCAS. (*Middle*) 2-HG concentrations. +/–, basal levels; ++, three- to fivefold; +++, 5- to 10-fold; ++++, 10- to 50-fold, relative to control (see Fig. S1). (*Bottom*) Western blots for IDH1$^{WT}$ and IDH1$^{R132H}$.

IDH1$^{R132H}$ showed marked elevation of 2-HG (Fig. 1*C*). Surprisingly, growth and clonogenicity of the cultures expressing human or mouse IDH1$^{R132H}$ were rescued to levels indistinguishable from parental cultures by introduction of vector encoding IDH1$^{WT}$, despite enhanced accumulation of 2-HG (Fig. 1 *B* and *C*). These findings are consistent with the suggestion that the growth deficit induced by mutant enzyme may be mediated by diversion of cytosolic α-ketoglutarate (α-KG) from IDH1$^{WT}$ (18), rather than by toxicity of 2-HG.

Previous studies have demonstrated that *IDH1*$^{R132H}$ human gliomas maintain normal concentrations of α-KG but have not identified a mechanism that compensates for diverted flux of α-KG into 2-HG (8, 9, 18, 19). In human tumors, mutations in *IDH1* and *IDH2* are mutually exclusive and invariably heterozygous (7). To examine whether *IDH1*$^{R132H}$ human gliomas up-regulate expression of WT IDH enzymes to negate growth-inhibitory effect of mutant enzyme, we compared expression profiling data from three series of histologically matched *IDH1*$^{R132H}$ and *IDH1*$^{WT}$ high-grade gliomas. Our analysis revealed no increase in mRNA signals for IDH1, IDH2, or IDH3 subunits in *IDH1*$^{R132H}$ tumors (Fig. 2*A*). Among probesets for which signal was most consistently up-regulated in *IDH1*$^{R132H}$ gliomas, the only probesets corresponding to enzymes were ones annotated as glutamate dehydrogenase 1 (GLUD1) and glutamate dehydrogenase 2 (GLUD2) (Fig. 2*A*). Analysis of RNA sequencing (RNAseq) data from The Cancer Genome Atlas (TCGA) GBM specimens confirmed that mRNA for both GLUD1 and GLUD2 are increased in *IDH1*$^{R132H}$ GBM relative to *IDH1*$^{WT}$ GBM (Fig. 2*B*). To determine whether GLUD1 or GLUD2 contributes to growth of *IDH1*$^{R132H}$ glioma, we examined effects of shRNA to GLUD1/2 on orthotopic grafts of an *IDH1*$^{R132H}$ human glioma line. Two shRNA targeting constructs to GLUD1/2 were used: sh647, which elicited partial reduction of GLUD1/2 protein, and sh662, which resulted in nearly complete elimination of detectable GLUD1/2 protein (Fig. 2*C*). Using T2-MRI to compare tumor volumes we found that grafts expressing sh647 or sh662 demonstrated a statistically significant reduction in tumor volume relative to grafts of cells transduced with control vector (Fig. 2*C* and Fig. S2). In addition, sh662-expressing grafts displayed reduction of apparent tumor cell density in H&E-stained sections (Fig. 2*C*). Consistent with growth effects of the targeting vectors, sh662, and to a lesser extent sh647, attenuated alterations in metabolite levels that distinguished tumors from host brain (Fig. 2*D* and Table S1). These results reveal a dependence of tumor growth on GLUD1 and/or GLUD2 but do not reveal the relative contributions of each of these highly homologous proteins to tumor growth.

GLUD1 and GLUD2 are mitochondrial enzymes that catalyze the conversion of glutamate to α-KG and lie immediately upstream from IDH1 and/or IDH2 in a reductive glutaminolysis pathway critical for lipogenesis and growth under conditions of hypoxia or mitochondrial dysfunction (20–22). To determine whether either GLUD1 or GLUD2 rescues growth-inhibitory effects of mutant IDH1 enzyme, we expressed each of these enzymes in glioma progenitor cells. Cultures of control murine glioma progenitor cells demonstrated robust immunoreactivity in Western blots using an antibody recognizing GLUD1 and GLUD2 (Fig. 3*A* and Fig. S3). Because *GLUD2* is a hominoid-specific gene, immunoreactivity in control murine glioma progenitor cells is presumed to reflect GLUD1 protein. Although expression of human GLUD1 did not promote growth of *IDH1*$^{R132H}$ murine glioma progenitors, GLUD2 rescued growth of these cultures (Fig. 3*A*). In contrast to the robust effects of GLUD2 on growth of cultures expressing *IDH1*$^{R132H}$, GLUD2 had no consistent effect on growth of parental cultures (Fig. 3*B*).

Because glutamate is a substrate for GLUD1 and GLUD2 and is a neurotransmitter abundant in brain extracellular space (23), we next sought to determine whether extracellular glutamate could influence growth. The addition of 20 μM glutamate to

A

| Probeset | Gene | MUT/WT | p value |
|---|---|---|---|
| 201193_at | IDH1 | 0.75 | 0.02 |
| 210046_s_at | IDH2 | 0.95 | .50 |
| 202070_s_at | IDH3A | 1.13 | .37 |
| 210418_s_at | IDH3B | 0.96 | .20 |
| 202471_s_at | IDH3G | 1.11 | .35 |
| 232833_at | NA | 4.1 | 8.9E-07 |
| 221824_s_at | MARCH8 | 2.3 | 3.2E-06 |
| 227719_at | SMAD9 | 2.3 | 6.5E-06 |
| 200946_x_at | GLUD1 | 2.4 | 1.1E-05 |
| 204100_at | THRA | 2.7 | 1.2E-05 |
| 205290_s_at | BMP2 | 3.5 | 1.9E-05 |
| 210753_s_at | EPHB1 | 2.3 | 2.0E-05 |
| 231131_at | FAM133A | 3.2 | 2.1E-05 |
| 215794_x_at | GLUD2 | 2.4 | 2.2E-05 |
| 239509_at | NA | 4.0 | 2.2E-05 |
| 230425_at | EPHB1 | 2.4 | 2.4E-05 |
| 215767_at | ZNF804A | 3.4 | 3.3E-05 |

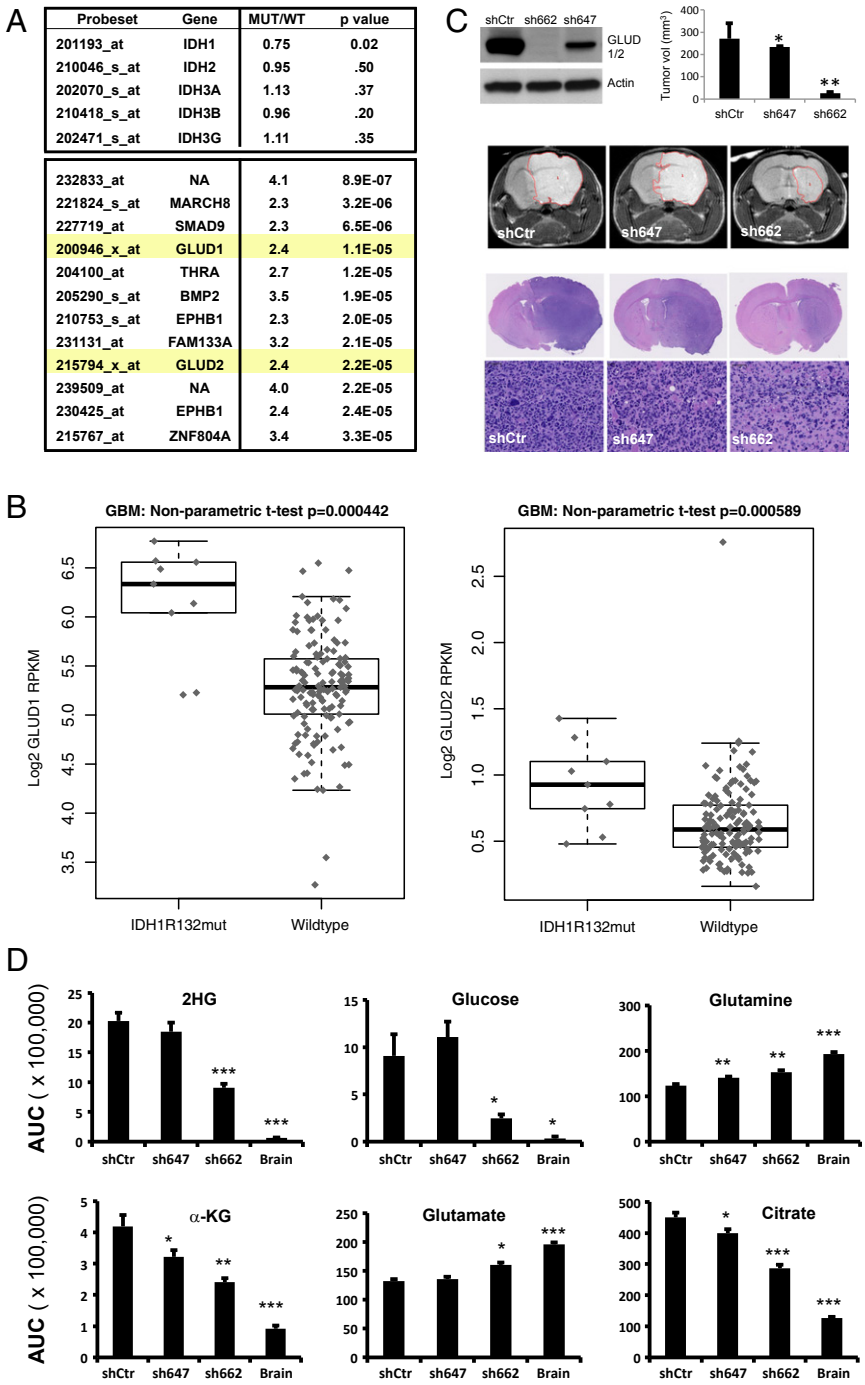


**Fig. 2.** GLUD1 and GLUD2 are overexpressed in *IDH1*$^{R132H}$ human GBM, and knockdown of GLUD1/2 inhibits orthotopic growth of an *IDH1*$^{R132H}$ glioma line. (*A*) List of all probesets showing twofold change and *P* value less than $1 \times 10^{-4}$ (*t* test) in each of three separate comparisons of *IDH1*$^{R132H}$ vs. *IDH1*$^{WT}$ high-grade glioma. Values reported represent mean fold change and *P* value for three comparisons. (*B*) RNAseq data for GLUD1 and GLUD2 comparing *IDH1*$^{R132H}$ vs. *IDH1*$^{WT}$ GBM. (*C*) Effects of shRNA to GLUD1/2 on orthotopic growth of *IDH1*$^{R132H}$ human glioma line BT142. (*Top Left*) Western blot showing effects of shRNAs on immunoreactivity for GLUD1/2. (See Fig. S3 for demonstration that GLUD1 and GLUD2 are both detected by Western blot). (*Top Right*) Volume (mean + SEM) of orthotopic BT142 grafts at 3 mo postimplantation. T2-weighted MRI. $*P < 0.05$, $**P < 0.0001$, with comparison with shCtr. (*Middle*) Representative T2 MRI images. Dotted line denotes tumor–brain boundary (Fig. S2). (*Bottom*) Images of H&E-stained sections of representative brains of grafted mice. (*D*) Concentrations of selected metabolite concentrations in IDH1$^{R132H}$ tumor grafts following GLUD1/2 knockdown. $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$, for comparison with shCtr, *t* test. AUC, area under the curve. (See Table S1 for all metabolites profiled.)

culture media enhanced growth of glioma progenitor cultures regardless of whether or not cultures had been engineered to express *IDH1*$^{R132H}$, shRNA to IDH1, GLUD1, or GLUD2 (Fig. 3*B*). These findings suggest that extracellular glutamate acts independently of IDH1 and GLUD2 enzymes to support growth.

Given previous reports that *IDH1*-mutant protein is deficient in reductive carboxylation (19) and that *IDH1*-mutant glioma is particularly sensitive to inhibition of glutaminase (24), we hypothesized that growth of *IDH1*$^{R132H}$ glioma progenitors may be limited by the ability to use glutamine as a carbon source for

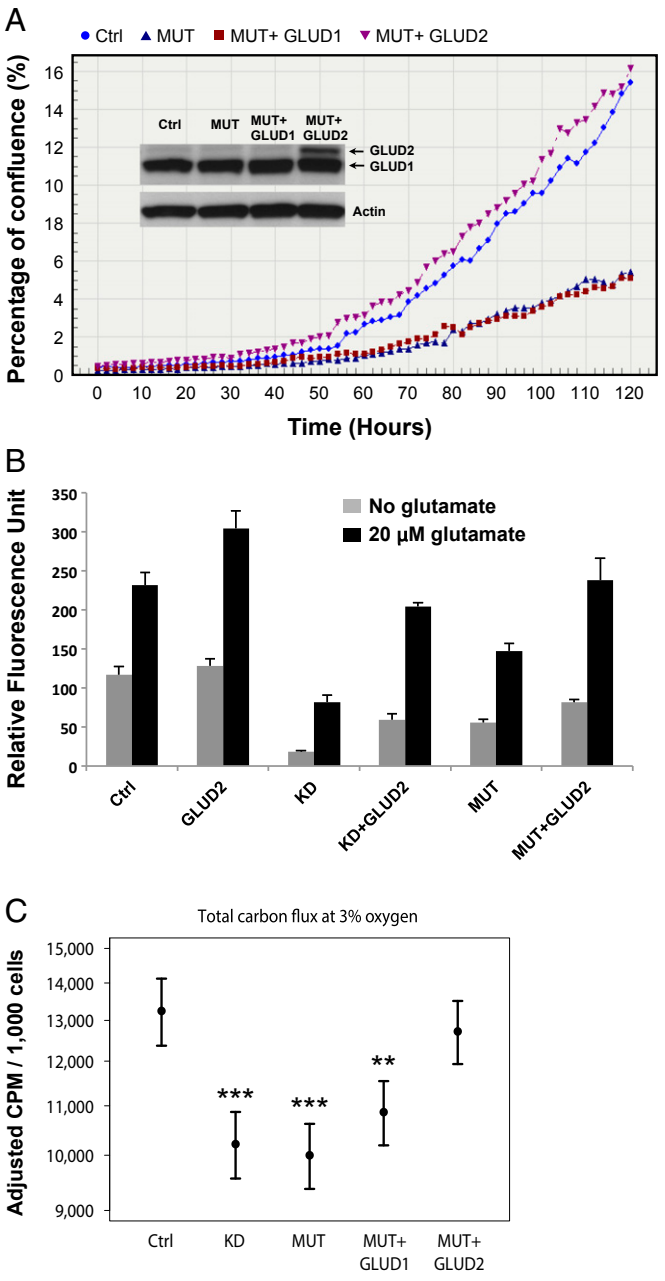


**Fig. 3.** GLUD2 promotes growth and metabolite flux to lipid in IDH1$^{R132H}$ glioma progenitors. In vitro behavior of uninfected glioma progenitor culture (Ctrl) was compared with the same line infected with RCAS vector(s) engineered to express murine IDH1$^{R132H}$ (MUT), human GLUD1 (GLUD1), GLUD2 (GLUD2), shRNA to murine IDH1 (KD), or sequential infections with MUT followed by GLUD1 or GLUD2. (*A*) IncuCyte growth assay and Western blot (*Inset*) using electrophoresis conditions optimized for separation of GLUD1 and GLUD2 (see *SI Methods* and Fig. S3). (*B*) CyQuant assay after 4 d of growth. For all conditions, $P < 0.05$ for 0 vs. 20 μM glutamate, *t* test. (*C*) Total carbon flux to lipids from glutamine and glucose with [$^{14}$C]glutamine or [$^{14}$C]glucose tracer for cultures maintained in standard conditions (3% oxygen). Means ± 95% confidence intervals, $n \geq 3$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control, mixed linear effects.

lipogenesis. Using [$^{14}$C]glucose or [$^{14}$C]glutamine to monitor carbon labeling from glucose or glutamine, we find that total fractional labeling from these metabolites into lipids was consistently reduced by either knockdown of endogenous *IDH1* or introduction of *IDH1*$^{R132H}$ (Fig. 3*C* and Fig. S4). Labeling of lipids in cultures of glioma progenitors expressing IDH1$^{R132H}$ was minimally influenced by introduction of human GLUD1. However, GLUD2 rescued flux in IDH1$^{R132H}$ cultures to levels similar to that of parental cultures (Fig. 3*C* and Fig. S4). Thus, growth effects exerted by IDH1$^{R132H}$ and GLUD2 in glioma progenitor cells are paralleled by alterations in fractional labeling of carbon derived from glucose or glutamine to lipids.

Given our finding that extracellular glutamate can support growth of glioma progenitors, we next sought to determine whether these cells use glutamate as a carbon source to support lipogenesis. Although incorporation of $^{14}$C from extracellular glutamate into lipids was detected in all cultures, only low levels of carbon flux to lipids were observed (Fig. S4), suggesting that additional mechanisms, such as extracellular signaling, may contribute to the effect of glutamate on cell growth.

We next sought to determine whether GLUD2 antagonizes growth-inhibitory effects of IDH1$^{R132H}$ in vivo. Using PDGF/RCAS to drive gliomas from *p53*$^{-/-}$ Nestin-tva glioma progenitor cultures, we observed that infection of cultures with IDH1$^{R132H}$/RCAS before implantation led to a marked prolongation in survival of engrafted mice (Fig. 4*A*). GLUD2/RCAS had no effect on outcome of mice engrafted with parental PDGF-driven gliomas; however, infection with GLUD2/RCAS completely abrogated the negative effect of IDH1$^{R132H}$ on tumor aggressiveness (Fig. 4*A*). Thus, in this model of in vivo glioma growth, GLUD2 exerts growth-promoting effects that are specific to tumors expressing IDH1-mutant protein. Mass isotopomer distribution analysis using $^{13}$C stable isotopes in the cultures used for grafting revealed evidence that introduction of GLUD2 into cells bearing *IDH1*$^{R132H}$ diverts glutamine-derived α-KG toward oxidative generation of citrate in the tricarboxylic acid (TCA) cycle of mitochondria at the expense of flux to 2-HG in the cytosol (Fig. 4*B* and Fig. S5).

Our findings suggest a model in which GLUD2 expression in human brain renders cells resistant to growth-inhibitory effects of *IDH1*$^{R132H}$ by supplying α-KG to fuel the citric acid cycle and support lipid synthesis (Fig. 4*C*). The relative importance of lipid synthesis vs. citric acid cycle in limiting growth of *IDH1*-mutant tumors cannot be determined from the current findings, nor can we eliminate other α-KG–dependent processes as underlying the growth-promoting effects of GLUD2 observed in the presence of mutant IDH1 enzyme. Restricted expression of GLUD2 (25) may provide a potential explanation for the tissue specificity of oncogenesis in patients with occurrence of *IDH1*$^{R132H}$ in multiple cell lineages; the majority patients with Ollier disease and Maffuci syndrome display evidence of early postzygotic occurrence of *IDH1*$^{R132H}$ mutation, yet glioma is the only malignancy occurring at increased frequency in these individuals (26, 27). Further, the absence of a *GLUD2* gene in nonhominoid species may account for the reported difficulties in identification or generation of animal models of *IDH1* mutant glioma (28, 29).

Although it is tempting to speculate that the growth-inhibitory effects of IDH1-mutant protein contribute to the better prognosis of *IDH1*-mutant human GBM, it is important to recognize that *IDH1*-WT and *IDH1*-mutant human GBMs reflect distinct disease entities that likely originate from separate cells of origin (5, 14) and, as such, numerous differences in biology likely contribute to differences in aggressiveness of the two tumor types. Our results do, however, underscore differences in metabolic vulnerability of human *IDH1*-mutant and *IDH1*-WT gliomas and suggest that therapeutic approaches targeting glutamate metabolism or availability of α-KG might be applicable to *IDH1*-mutant gliomas.

Although previous work implicates glutamate dehydrogenase as a regulator of GBM growth (30), our findings reveal an unexpected lack of redundancy between the capabilities of GLUD1 and GLUD2. Although our results do not allow us to determine whether GLUD1 contributes to growth of IDH1-mutant cells, they do clearly reveal that GLUD2 promotes growth of IDH1-mutant cells in a manner that is not duplicated by overexpression of GLUD1. Whether the unique actions of GLUD2 observed

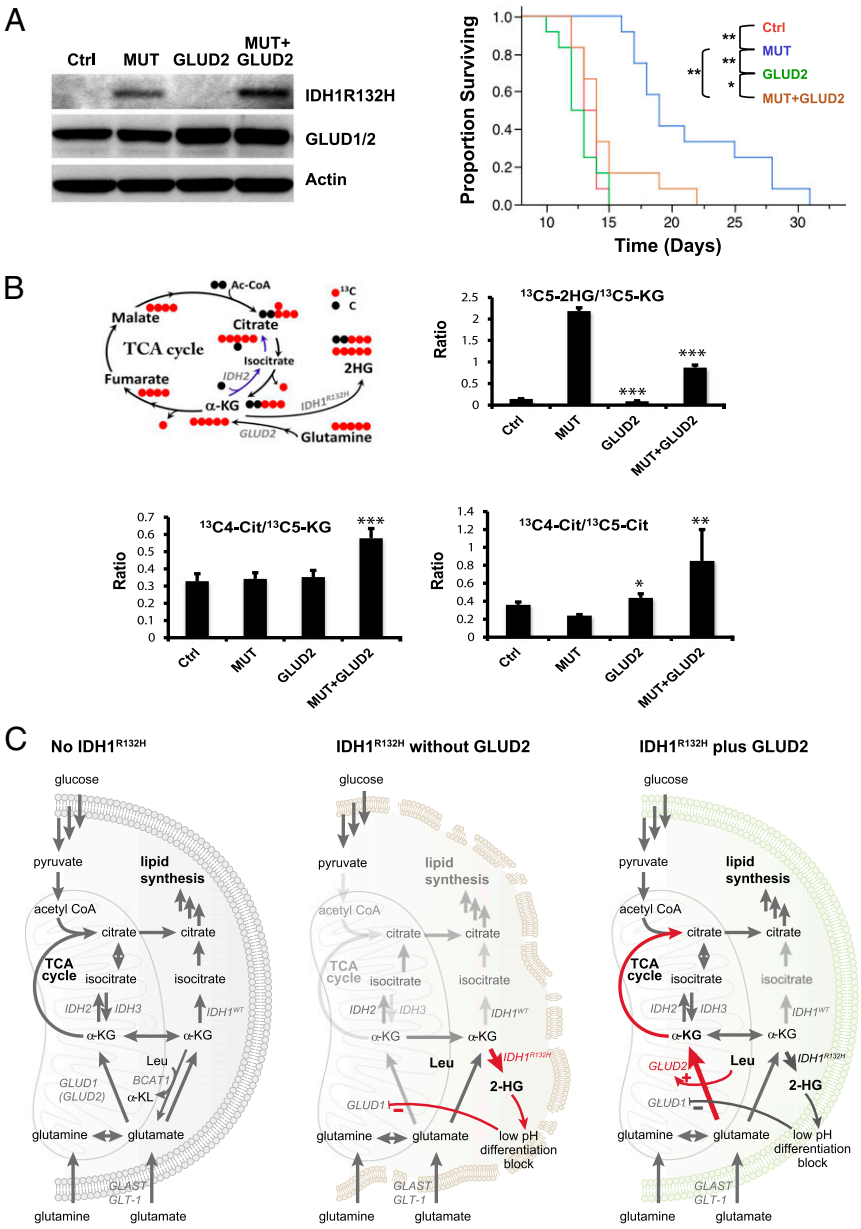


**Fig. 4.** GLUD2 rescues in vivo growth of murine gliomas expressing IDH1$^{R132H}$. (*A*) Murine glioma progenitors driven to form tumors by human PDGFB/RCAS were treated with no additional vector (Ctrl), IDH1$^{R132H}$/RCAS (MUT), GLUD2/RCAS (GLUD2), or IDH1$^{R132H}$/RCAS followed by GLUD2/RCAS (MUT + GLUD2). (*Left*) Western blot for IDH1$^{R132H}$ and GLUD1/2. (*Right*) Survival of mice bearing grafts of the engineered cells. *$P < 0.05$, **$P < 0.001$ vs. Ctrl, log rank. (*B*) Effects of GLUD2 on ratios of metabolites derived from $^{13}$C5 glutamine. (*Upper Left*) Schematic diagram of carbon flux in TCA cycle starting from $^{13}$C5–α-KG. (*Upper Right*) Decreased 2-HG production from glutamine-derived α-KG. (*Lower Left*) Increased citrate production from TCA cycle. (*Lower Right*) Increased citrate production through oxidative phosphorylation over through reductive glutamine metabolism. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for GLUD2 effect compared with Ctrl (for GLUD2) or MUT (for MUT + GLUD2). (*C*) Model of proposed actions of GLUD2 to rescue growth of glioma progenitors expressing IDH1$^{R132H}$. The arrows in red highlight the effects of IDH1$^{R132H}$ (*Center*) and of GLUD2 in the presence of IDH1$^{R132H}$ (*Right*).

are due to subcellular localization or biochemical properties of the enzyme cannot be determined from the current findings. Intriguingly, the *GLUD2* gene is unique to hominoids and has undergone rapid evolutionary selection concomitant with expansion of prefrontal cortex (25, 31), the site at which *IDH1$^{R132H}$* glioma most frequently occurs (14). The prevailing belief is that selection pressure during hominoid evolution for higher flux of glutamate transmitter in prefrontal cortex led to optimization of GLUD2 for degradation of glutamate in the nervous system environment. We speculate that the amino acid substitutions in GLUD2 that confer lower pH optimum and lack of negative regulation by GTP not only optimize the enzyme's ability to support glutamate transmitter flux in the normal human brain (25, 31) but also account for the ability of this enzyme to support growth of *IDH1$^{R132H}$* glioma progenitors. In particular, we point to the likelihood that the pH optimum of GLUD2 is better suited than that of GLUD1 for glioma progenitors acidified by high intracellular 2-HG. Of note, the recent finding that *IDH1$^{R132H}$* GBMs lack expression of Branched Chain Amino acid Transaminase-1 (BCAT1) (15) offers a mechanism to sustain high intracellular concentrations of both α-KG, a product of GLUD2, and leucine, an activator of GLUD2 (25).

In addition to revealing a role for GLUD2 in supporting growth of *IDH1$^{R132H}$* glioma progenitor cells, our results point to

the likelihood that extracellular glutamate resulting from neurotransmitter release also contributes to growth of *IDH1*$^{R132H}$ glioma. Thus, metabolic specialization in both tumor cell-of-origin and the stromal niche seems to contribute to vulnerability of human forebrain to formation and growth of *IDH1*$^{R132H}$ glioma, raising the possibility that adaptations that facilitate human cognition may have come at the cost of increased susceptibility to this tumor type.

## Methods

Glioma progenitor cultures were established from brains of newborn mice resulting from breedings of Nestin-tva-TG.*p53*$^{+/-}$.B6 × Nestin-tva-TG.*p53*$^{-/-}$.B6 adults. Cultures were created by dissociation of whole forebrain and maintained in neurosphere media in 5% $CO_2$ and 3% $O_2$, unless otherwise indicated. With the exception of the experiment reported in Fig. 1*A*, a single Nestin-tva/*p53*$^{-/-}$ culture (line 5) was the parental culture used for all experiments using glioma progenitors. Glioma progenitor cultures were engineered to express proteins or shRNA constructs by in vitro infection with appropriate RCAS vectors. Bulk culture growth and clone formation assays were conducted in 3% oxygen and monitored by CyQuant and microscopic inspection, respectively. In addition, culture growth in 20% oxygen was monitored by CyQuant or Incucyte. Concentrations of 2-HG in culture supernatants and cell pellets were determined by liquid chromatography coupled with tandem mass spectrometry. For in vivo glioma growth studies, $5 \times 10^5$ mouse glioma progenitor cells infected in vitro with RCAS vectors or $1 \times 10^5$ cells of human heterozygous *IDH1*$^{R132H}$ line BT142 (SRC-4002; American Type Culture Collection) infected in vitro with lentiviral vectors were implanted into right striatum of female CD1 nude mice or NOD/SCID mice. Protocols for all in vivo studies were reviewed and approved by the Genentech Institutional Animal Care and Use Committee.

Two replicate in vivo experiments were conducted with IDH1$^{R132H}$ and GLUD2 expression in murine glioma progenitor grafts and a single in vivo experiment was conducted with GLUD1/2 shRNA knockdown in the human BT142 line. Tumor growth was monitored by host survival (murine grafts) or T2-weighted MRI (BT142) and, in all experiments, presence or absence of tumor was verified by histological examination. Expression of mRNA differences between *IDH1*$^{R132H}$ and *IDH1*$^{WT}$ human gliomas was determined by analysis of microarray data generated in previous studies (14, 32) from two sets of GBM and one set of grade III astrocytoma as well as from TCGA RNAseq data from GBMs.

See *SI Methods* for detailed methods.

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