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Lactate dehydrogenase A silencing in IDH mutant gliomas

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Background. Mutations of the isocitrate dehydrogenase 1 and 2 gene (*IDH1/2*) were initially thought to enhance cancer cell survival and proliferation by promoting the Warburg effect. However, recent experimental data have shown that production of 2-hydroxyglutarate by IDH mutant cells promotes hypoxia-inducible factor (HIF)1α degradation and, by doing so, may have unexpected metabolic effects.

Methods. We used human glioma tissues and derived brain tumor stem cells (BTSCs) to study the expression of HIF1α target genes in IDH mutant (^{mt}) and IDH wild-type (^{wt}) tumors. Focusing thereafter on the major glycolytic enzyme, lactate dehydrogenase A (*LDHA*), we used standard molecular methods and pyrosequencing-based DNA methylation analysis to identify mechanisms by which *LDHA* expression was regulated in human gliomas.

Results. We found that HIF1α-responsive genes, including many essential for glycolysis (*SLC2A1*, *PDK1*, *LDHA*, *SLC16A3*), were underexpressed in IDH^{mt} gliomas and/or derived BTSCs. We then demonstrated that *LDHA* was silenced in IDH^{mt} derived BTSCs, including those that did not retain the mutant IDH1 allele (mIDH^{wt}), matched BTSC xenografts, and parental glioma tissues. Silencing of *LDHA* was associated with increased methylation of the *LDHA* promoter, as was ectopic expression of mutant IDH1 in immortalized human astrocytes. Furthermore, in a search of The Cancer Genome Atlas, we found low expression and high methylation of *LDHA* in IDH^{mt} glioblastomas.

Conclusion. To our knowledge, this is the first demonstration of downregulation of *LDHA* in cancer. Although unexpected findings, silencing of *LDHA* and downregulation of several other glycolysis essential genes raise the intriguing possibility that IDH^{mt} gliomas have limited glycolytic capacity, which may contribute to their slow growth and better prognosis.

Keywords: gliomas, glycolysis, IDH mutation, LDHA, methylation.

Deregulation of oncogenes and tumor suppressor genes is known to affect the expression and activity of transporters, sensors, and enzymes that regulate metabolism,[1] but actual mutations in genes directly involved in metabolism, such as fumarate hydratase and succinate dehydrogenase, which are members of the tricarboxylic acid cycle, have only recently been demonstrated.[2] Not surprisingly, then, the discovery of mutations of isocitrate dehydrogenase 1 and 2 (*IDH1/2*), first in colon cancer and soon thereafter in different subtypes of gliomas, including oligodendrogliomas, astrocytomas, mixed oligoastrocytomas, and secondary glioblastomas,[3,4] ignited a feverish interest in cancer cell metabolism. Initially, IDH1/2 mutations were anticipated to directly impact cellular metabolism and promote aerobic glycolysis, the metabolic pathway favored by cancer cells to support rapid proliferation.[5,6] However, despite advances in the understanding of the function of the mutant IDH enzyme,[7–10] the hypothesis that IDH1/2 mutations directly alter cancer cell metabolism remains unproven.

2-Hydroxyglutarate (2-HG), the product of the neomorphic activity of the mutant IDH1/2 enzymes, competitively inhibits α-ketoglutarate–dependent enzymes,[11] including histone demethylases and 5-methylcytosine hydroxylases that are essential for epigenetic regulation of gene expression.[12–14] Consequently, via 2-HG–dependent epigenetic remodeling, IDH mutations may interfere with differentiation, proliferation, survival, and metabolism.[6] 2-Hydroxyglutarate also affects the activity of *EGLN* prolyl 4-hydroxylases (PHDs), which are responsible for oxygen-dependent

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degradation of hypoxia-inducible factor (HIF)1α. Although stabilization of HIF1α by 2-HG has been reported,[15] a recent study has clarified that 2-HG stimulates PHD activity, leading to the degradation of HIF1α.[16] The subsequent downregulation of HIF1α-responsive genes by 2-HG may have unique consequences for human cancer cells, including limitation of the metabolic shift toward aerobic glycolysis, the so-called Warburg effect.

A key step in glycolysis is the conversion of pyruvate to lactate, catalyzed by the lactate dehydrogenase (LDH) complex, one subunit of which is *LDHA*. LDHA enhances the efficiency of the LDH complex, allowing the rapid flux through glycolysis that is necessary to meet the energy needs of rapidly proliferating cells. As such, LDHA is considered a major biomarker of glycolytic activity.[17–20] *LDHA* contains HIF1α binding sites in its promoter and is induced under hypoxic conditions, allowing normal cells to switch to an oxygen-independent glycolytic metabolic phenotype when deprived of oxygen.[21,22] *LDHA* is overexpressed in cancer cells, and silencing of *LDHA* typically results in accelerated oxygen consumption, increased apoptosis, decreased proliferation, and strong inhibition of tumorigenicity.[23–27]

Here, we report that multiple HIF1α-responsive genes necessary for glycolysis are underexpressed in IDH mutant (^{mt}) gliomas and brain tumor stem cells (BTSCs) derived from IDH^{mt} tumors, including human BTSCs that have lost the mutant *IDH1* allele and no longer produce 2-HG. Due to its central role in glycolysis, we focused our attention on *LDHA*. Our data suggest that *LDHA* is silenced through IDH^{mt}-dependent methylation of its promoter. Silencing of *LDHA* and downregulation of other glycolytic genes is a surprising finding in the context of human cancer cells but may help to explain the slower progression and better prognosis of IDH^{mt} gliomas.

Materials and Methods

Glioma Samples and BTSC Culture

Tissue samples were obtained through the University of Calgary Neurologic and Pediatric Tumor and Related Tissue Bank, following informed consent from glioma patients during their operative procedures and approval by the University of Calgary Ethics Review Board. BTSC lines were cultured in nonadherent, serum-free conditions as previously described.[28,29]

Microarray

RNAs were extracted from lines and tumor tissues and the quality confirmed with a Bioanalyzer (Agilient). Reverse transcription polymerase chain reaction was performed on high-quality RNAs, and cDNAs were used on an HT12-v4 microarray (Ilumina). The data were analyzed using Chipster software. Student's *t*-test was performed on normalized gene expression data to discern differentially expressed genes in IDH^{mt} versus IDH wild-type (^{wt}) gliomas and derived BTSC lines ($P < .05$). Clustering was performed on genes selected for differential expression between IDH^{mt} and IDH^{wt} samples. Samples and selected genes were clustered using Pearson correlation as a distance measure and average linkage for constructing the dendrogram.

Real-time Quantitative PCR

RNAs were extracted from lines and tissues using the RNeasy kit (Qiagen) following manufacturer's instructions. Genomic DNA elimination was performed using Qiagen gDNA eliminator columns. Quality RNAs were reverse transcribed using the Sensiscript Kit (Invitrogen), and cDNAs were used for quantitative (q)PCR using a Taqman gene expression assay as instructed (*LDHA* Hs00855332_g1, cat. #4331182, FAM, Applied Biosystems).

Immunoblotting

Frozen patient tumor tissues, tumor xenografts, normal brain from nonobese diabetic severe combined immunodeficient mice, and BTSC lines were lysed in radioimmunoprecipitation assay buffer (50 mM Tris, 150 mM NaCl, 0.1% sodium dodecyl sulfate [SDS], 0.5% Na deoxycholate, and 1% nonyl phenoxypolyethoxylethanol) and Complete Protease Inhibitor Cocktail Tablets (Roche). Each protein lysate (20 μg) was separated by SDS–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (standard protocol). Membranes were blocked in Tris-buffered saline with 5% nonfat dry milk and incubated for 1 h with a mouse monoclonal antibody to LDHA (1:1000; sc-137243, Santa Cruz Biotech) specific for an epitope mapping between amino acids 6 and 42 at the N-terminus of human LDHA and goat antihuman actin antibodies (1:500 and 1:2000; Santa Cruz Biotech), followed by donkey antimouse and antigoat horseradish peroxidase–conjugated secondary antibodies (1:5000; Millipore).

IDH Sequencing and Copy Number Analysis

Genomic DNA was extracted from lines and tumors using DNeasy (Qiagen) following the manufacturer's instructions. DNAs were submitted to PCR to amplify exon 4 of *IDH1* and exon 2 of *IDH2*, as described elsewhere,[3,4] and 2 μg of BTSC and tumor DNAs were sent to Expression Analysis.

Gas Chromatography Mass Spectrometry Experimentation

Fifty-microliter aliquots of conditioned cell media were extracted and analyzed with a Waters GCT Premier mass spectrometer using parameters as follows: injector temperature of 275°C, He carrier gas flow rate of 1.2 mL/min, DB5-MS column (30 m × 0.25 mm × 0.25 μm; Agilent), gas chromotography (GC) oven ramp (12°C/min) from 80°C to 320°C followed by 8 min hold at 320°C. Ions between m/z 50 and 800 were collected and analyzed. The 2-HG levels were normalized with total ions and determined using 2-HG ions m/z 349, 247, and 203.

Pyrosequencing-based Cytosine–Phosphate–Guanine Methylation Analysis

Genomic DNA was isolated and submitted to EpigenDX for pyrosequencing-based cytosine–phosphate–guanine methylation analysis. An analyzed sequence in the LDHA promoter is shown in Supplementary Fig. 1.

Immortalized Normal Human Astrocyte Model

Immortalized human astrocytes were created by serial introduction of retroviral constructs encoding human telomerase reverse transcriptase and human papillomavirus 16 E6/E7 (to inactivate both p53 and pRb) in normal human astrocytes (NHAs) followed by drug selection as previously described.[30] Immortalized human astrocytes expressing exogenous wild-type or mutant IDH were created by further introduction of a lentiviral construct containing cDNAs for green fluorescent protein and either a human IDH1 cDNA (MHS1010-58017, Open Biosytems, Thermo Scientific) or an IDH cDNA generated by site-directed mutagenesis to encode the R^{132H} IDH (Quick Change II, Applied Biosystems).

^{1}H Magnetic Resonance Spectroscopy of Cell Lysates

Proton magnetic resonance spectroscopy (^{1}H MRS) was used to detect and quantify the intracellular level of 2-HG in the 2 NHA cell lines. To do

so, 1.5×10^8 cells were extracted using the dual-phase extraction method.[31,32] Proton MRS spectra of IDH^{mt} NHA ($n = 3$) and IDH^{wt} NHA ($n = 3$) cell extracts were acquired on a 14 T Bruker BioSpin spectrometer equipped with a 5-mm broadband probe using the following acquisition parameters: 90-degree flip angle, repetition time 2 s, spectral width 7194 Hz, 20 000 points, number of transients 100. Spectral assignments for 2-HG were based on literature reports (eg, www.hmdb.ca), 2-HG levels were quantified by deconvolution of peaks in ACD/SpecManager 9 (Advanced Chemistry Development), and correction for saturation and normalization was set to cell number and to an external reference of known concentration (3-(trimethylsilyl)-2,2,3,3-tetradeuteropropionic acid; Cambridge Isotope Laboratories).

Public Database Analysis

LDHA expression and de-identified survival data for glioma patients were obtained from the Repository of Molecular Brain Neoplasia Data (REMBRANDT; https://caintegrator.nci.nih.gov/rembrandt/ (last accessed December 10, 2013)). *LDHA* over- or underexpression was defined as a 2.0-fold change. *LDHA* expression, methylation, gene expression subtypes, and IDH1 mutation data in glioblastoma multiforme (GBM) were obtained from The Cancer Genome Atlas (TCGA) database. Only GBM samples with sequencing, methylation, and expression data available were used (TCGA GBM gene expression [AffyU133a], DNA methylation [Illumina HumanMethylation27, probe IDs: cg01316516 and cg20429911; Supplementary Fig. 1A and C], and somatic mutation, $n = 126$).

Statistics

We used 2-tailed Student's *t*-tests, a 1-way ANOVA with Bonferroni posttest to compare all pairs of columns, and Mann–Whitney *U* -tests.

Results

IDH Mutant Gliomas and Derived BTSCs Underexpress HIF1α-Responsive Genes Including Genes Essential for Glycolysis

To assess differences in gene expression between IDH^{mt} and IDH^{wt} gliomas, including differential expression of HIF1α-responsive genes, we performed a microarray analysis on glioma tissues of different histological subtypes and World Health Organization grades and sequenced *IDH1* and *2*. Eighty-seven validated HIF1α targets (Supplementary Table 1) were assessed for differential expression. HIF1α-responsive genes were globally underexpressed in IDH^{mt} glioma tissues relative to IDH^{wt} gliomas (Fig. 1A); furthermore, several were glycolysis-related genes (*PGK1, PKM2, LDHA, SLC16A3,* and *CA9*). Likewise, under hypoxic conditions, IDH^{mt} derived BTSCs (BT054, BT142, and BT088) underexpressed HIF1α-responsive genes relative to IDH^{wt} derived BTSCs (Fig. 1B), most of which are essential for glycolysis (*SLC2A1, HK2, PDK1, LDHA, SLC16A3, ENO1,* and *CA9*). HIF1α target genes not presented in the heatmaps were not significantly differentially expressed between IDH^{wt} and IDH^{mt} samples. These results suggest that IDH^{mt} gliomas underexpress genes downstream of HIF1α, including genes essential for cells that rely heavily on glycolysis to support their rapid growth.

Subsequently, we focused our attention on *LDHA* because it was among the most downregulated genes in IDH^{mt} gliomas and derived BTSC lines, and because of its role as a major checkpoint in the glycolytic switch. Of note, using the REMBRANDT public database, we showed that patients whose gliomas underexpressed *LDHA* (>2-fold) had a median survival of over 50 months compared

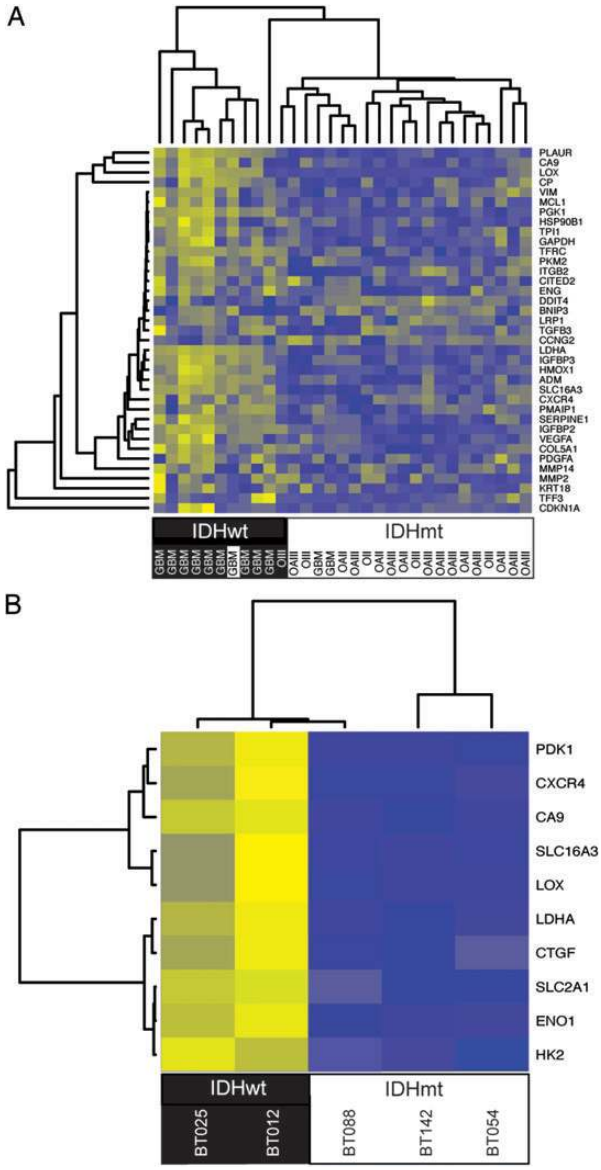

Fig. 1. HIF1α-responsive genes, including glycolysis-related genes, are underexpressed in IDH^{mt} gliomas and BTSCs. (A) Heatmaps showing differentially expressed genes among 87 validated HIF1α target genes (cutoff, *t*-test: $P < .05$) in IDH^{mt} vs IDH^{wt} gliomas and (B) derived BTSC lines cultured under hypoxic conditions for 2 weeks (blue, lower expression; yellow, higher expression). OAIII, oligoastrocytoma grade III; OIII, oligodendroglioma grade III.

with 16 months for those whose tumors overexpressed *LDHA* ($P < .01 \times 10^{-6}$; Fig. 2). Although LDHA expression can directly influence tumor aggressiveness, and hence survival, it appears here that LDHA expression may follow IDH mutation status, which in turn influences survival.

LDHA Is Underexpressed in IDH^{mt} Derived BTSCs, Xenografts, and Parent Tumors

Real-time qPCR confirmed that *LDHA* was highly underexpressed in BTSC lines that were derived from IDH^{mt} gliomas (BT054, BT088,

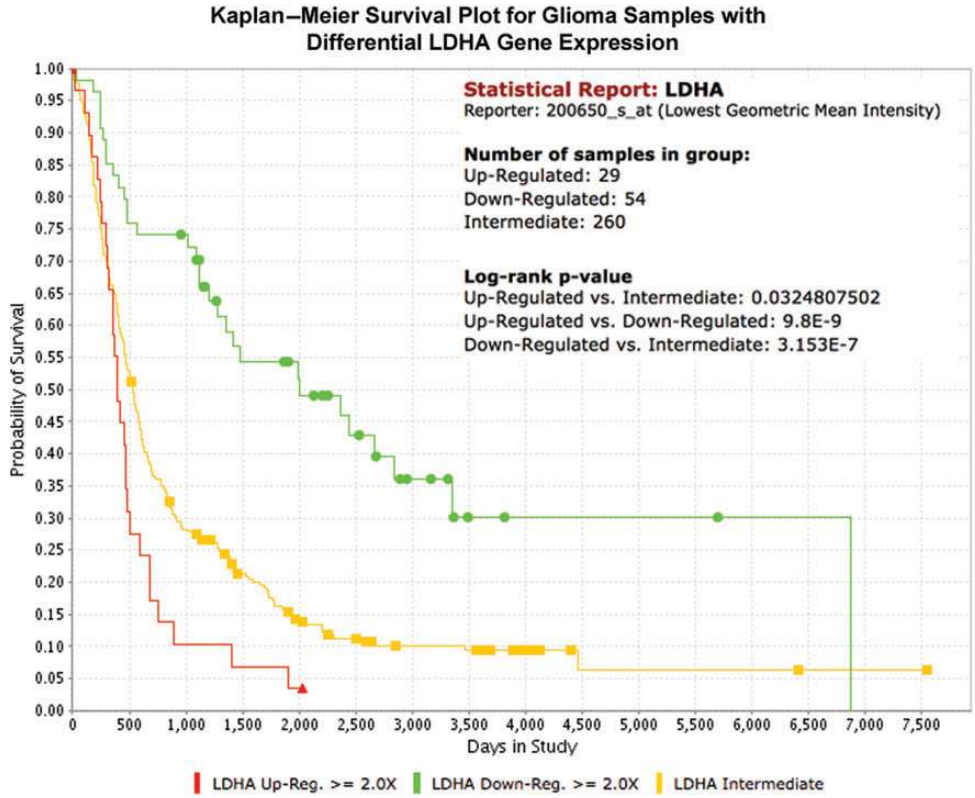

Fig. 2. *LDHA* expression correlates with survival. Kaplan–Meier survival plot according to differential *LDHA* expression (cutoff >2-fold; REMBRANDT).

BT092, BT142) compared with those derived from IDH^{wt} gliomas, even when grown under hypoxic culture conditions (Fig. 3A). At the protein level, LDHA was undetectable in the 4 BTSC lines derived from IDH^{mt} gliomas but strongly expressed in 32 of 34 lines (94%) derived from IDH^{wt} tumors (Fig. 3B–D). Similarly, LDHA protein was undetected in orthotopic xenografts of BTSCs derived from IDH^{mt} gliomas (Fig. 3E). Finally, LDHA was also underexpressed in IDH^{mt} parent tumors compared with IDH^{wt}; differences in LDHA expression were less striking in tumors than in BTSCs, perhaps due to admixed normal cells (Fig. 3F).

BTSCs That "Shed" the Mutant IDH1 Allele Do Not Produce 2-HG

Two of the BTSC lines in the study, BT088[29] and BT092, were derived from IDH^{mt} gliomas but were determined by sequencing to be IDH^{wt} (Fig. 4A). Single nucleotide polymorphism analyses demonstrated that both of these IDH^{mt} tumor-derived lines had lost all (or part) of one copy of chromosome 2q and retained a single *IDH1* allele, which was confirmed by sequencing to be the wild type (Fig. 4B). As a consequence of loss of the mutant allele, these mutant-derived IDH^{wt} BTSC lines (mIDH^{wt}) no longer produced 2-HG (Fig. 4C). IDH mutation has been shown to promote HIF1α degradation,[16] explaining downregulation of *LDHA* and other HIF1α target genes in IDH^{mt} gliomas. To this important finding, we now add the further observation that *LDHA*, and possibly other HIF1α-responsive genes involved in glycolysis, continue to be highly underexpressed in BTSC lines derived from IDH^{mt} gliomas, whether or not they retain the mutant allele and independently of continued 2-HG production.

LDHA Is Silenced by Promoter Methylation in IDH^{mt} and mIDH^{wt} BTSC Lines

Degradation of HIF1α in IDH^{mt} cells is dependent on 2-HG production,[16] yet mIDH^{wt} BTSCs fail to reexpress *LDHA* even under hypoxic conditions. This led us to hypothesize that a mechanism independent of HIF1α regulates *LDHA* expression in IDH^{mt} gliomas and derived BTSCs. Since 2-HG production alters the epigenome,[13] we inquired whether silencing of *LDHA* was an epigenetic phenomenon persisting after loss of the mutant *IDH1* allele. This idea is supported by a recent report showing that specific inhibition of IDH mutant enzyme can reverse the IDH^{mt}-dependent histone methylation profile but not IDH^{mt}-dependent DNA methylation, which is more stable.[33] Accordingly, DNA samples from BTSCs were submitted to bisulfite treatment and pyrosequencing. The *LDHA* promoter was heavily methylated in IDH^{mt} and mIDH^{wt} BTSC lines compared with IDH^{wt} BTSC lines, where little methylation was seen (Fig. 5A, Supplementary Fig. 1B).

To determine if *IDH1* mutation alone could mediate *LDHA* silencing by promoter methylation, we infected immortalized NHAs with lentiviral particles containing either the *IDH1*^{wt} or *IDH1*^{mt} construct. Western blotting and MRS confirmed the expression of the mutant IDH enzyme and 2-HG production in IDH^{mt} NHAs (Fig. 5B), respectively. We then showed that ectopic expression of the mutant enzyme in NHAs led to increased methylation of the

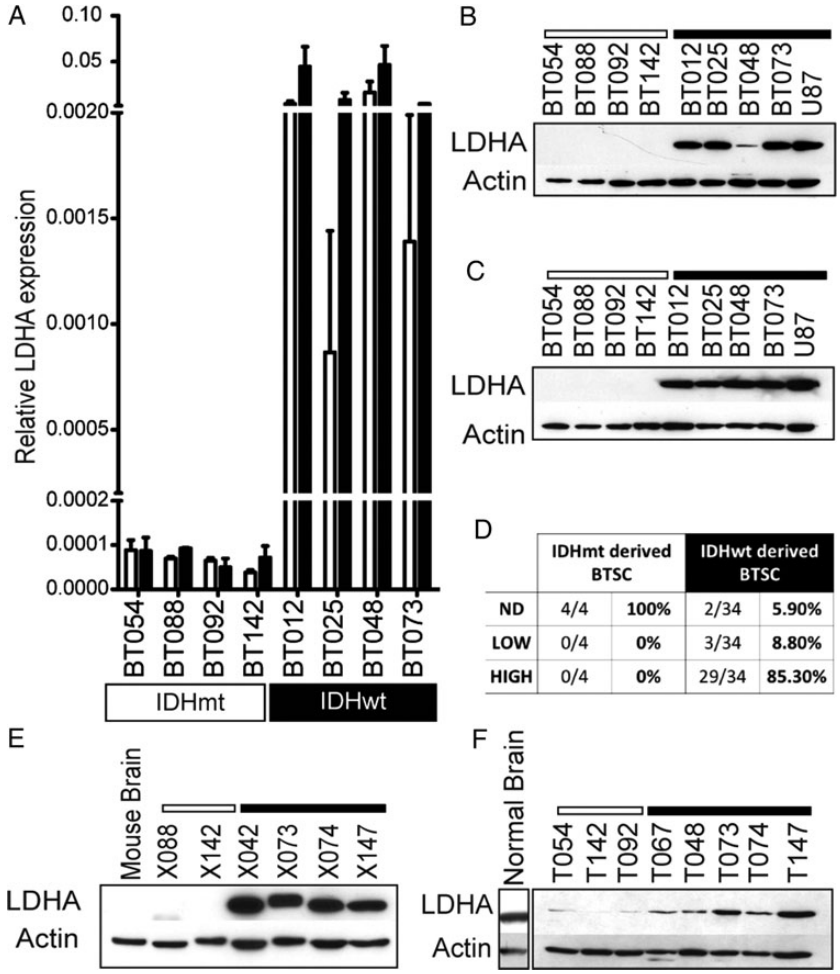

Fig. 3. *LDHA* expression in IDH^{wt} vs IDH^{mt} gliomas. (A) *LDHA* expression by RT-qPCR in BTSCs cultured under normoxia (white) and hypoxia (1% for 48 h, black). Western blot performed on BTSC lines (black: IDH^{wt} derived lines; white: IDH^{mt} derived lines) cultured under (B) normoxia and (C) hypoxia (1% O_2). (D) Table summarizing the detection of *LDHA* by Western blot in 38 BTSC lines. Western blot performed on (E) xenografts (white: IDH^{mt} derived; black: IDH^{wt} derived) and (F) originating tumor samples (black: IDH^{wt}; white: IDH^{mt}).

LDHA promoter compared with IDH^{wt} NHAs. Although methylation of the promoter increased significantly ($P < .02$), it was insufficient to alter LDHA expression (Fig. 5C). Interestingly, despite the increase in methylation compared with control NHAs, the degree of promoter methylation was lower in IDH^{mt} NHAs than in IDH^{wt} BTSC lines, raising the possibility that additional time in culture may be necessary for IDH^{mt} NHAs to acquire sufficient methylation marks to result in an observable difference in *LDHA* expression due to promoter silencing. Turcan et al[34] previously reported that >30 passages were required for IDH^{mt} NHAs to acquire the hypermethylated phenotype. Here we tested lower numbers of passages (10–15 IDH^{mt} NHAs). However, our findings support a direct link between IDH mutation and increased methylation of the *LDHA* promoter. Of note, in the Turcan study, *LDHA* was one of the preferentially methylated genes in IDH^{mt} NHAs.[34]

LDHA Is Underexpressed and Highly Methylated in IDH^{mt} Gliomas

To further explore the associations among IDH status, *LDHA* expression, and *LDHA* promoter methylation in a more clinical setting, we examined these relationships using glioma tissues. We found that *LDHA* was underexpressed in IDH^{mt} gliomas compared with IDH^{wt} tumors (Fig. 6A). IDH^{wt} gliomas, like normal brain tissue, had lower levels of *LDHA* promoter methylation than IDH^{mt} gliomas, which displayed high methylation of the *LDHA* promoter (Fig. 6B, Supplementary Fig. 1B). Of note, there was no significant association between *LDHA* promoter methylation and tumor type or grade (Fig. 6C). We further confirmed these results using the larger and publicly available TCGA database. Significant LDHA underexpression and high LDHA promoter methylation were observed in IDH^{mt} GBM compared with IDH^{wt} GBM independently of the gene expression subtypes (Fig. 6D and E, Supplementary Fig. 1C). These results demonstrate that LDHA is downregulated and LDHA promoter highly methylated in IDH^{mt} gliomas with no association with glioma type, grade, or gene expression subtype.

Discussion

We have shown that IDH^{mt} gliomas and BTSCs derived from IDH^{mt} tumors underexpress HIF1α-responsive genes, including several that are critical for glycolysis, and are typically overexpressed in

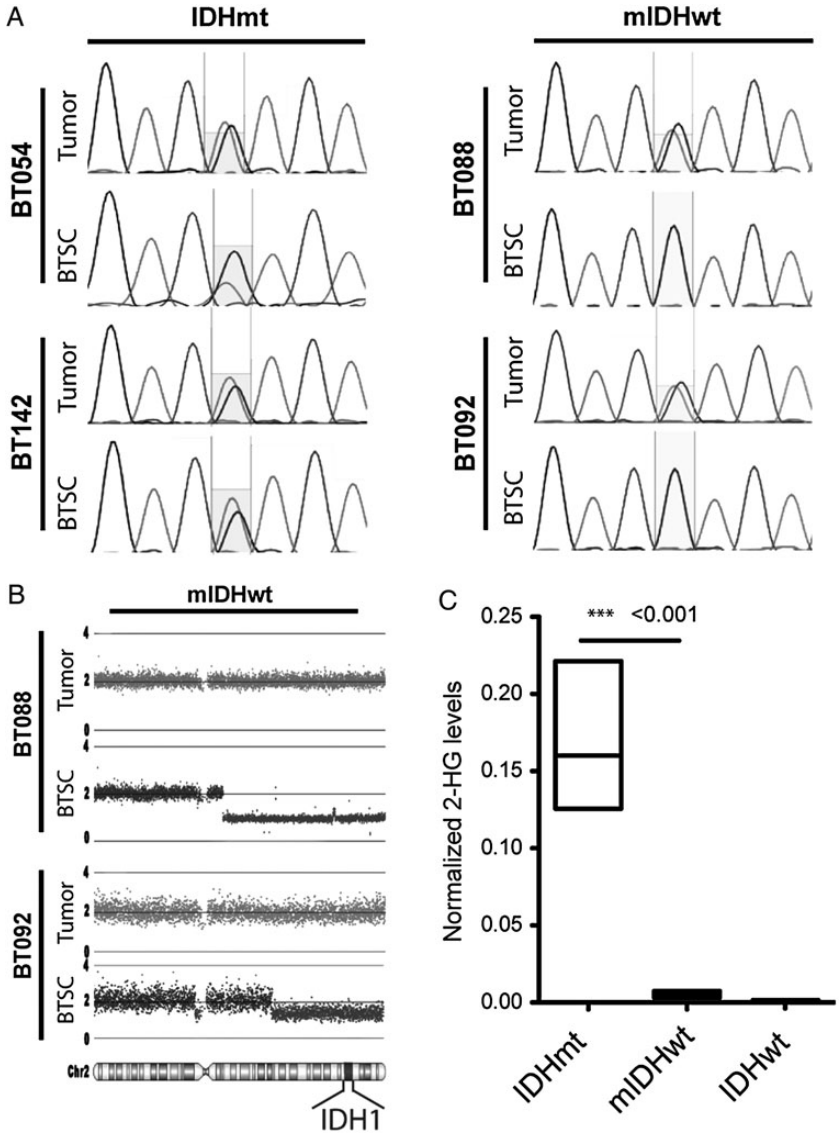

Fig. 4. Loss of IDH mutation: mIDH^{wt} BTSC lines. (A) *IDH1* sequencing on DNA extracts from tissue samples and matching BTSC lines. (B) Copy number analysis of chromosome 2 including *IDH1* locus region (2q33.3). (C) Detection of 2-HG by GC mass spectrometry in media conditioned by the IDH^{mt} line BT054, the mIDH^{wt} line BT088, and the IDH^{wt} line BT012.

glycolytic cancer cells. Furthermore, we have shown that *LDHA* is silenced in IDH^{mt} BTSC lines, IDH^{mt} BTSC xenografts, and IDH^{mt} gliomas of different types and grades. Interestingly, *LDHA* silencing persists in IDH^{mt} derived BTSC lines in the absence of continued production of 2-HG, a finding suggesting that silencing of *LDHA* is not solely due to 2-HG–dependent degradation of HIF1α.[16] IDH^{mt} and mIDH^{wt} BTSCs as well as IDH^{mt} glioma tissue samples also harbor a highly methylated *LDHA* promoter. Moreover, increased methylation of the *LDHA* promoter occurs in immortalized NHAs expressing the mutant IDH1 enzyme, thus demonstrating a direct link between IDH mutation and *LDHA* promoter methylation. Together, these results suggest that *LDHA* is silenced by IDH^{mt}-dependent promoter methylation. However, the fact that DNA methylation is not necessarily the first step in gene silencing but rather a mechanism for permanently silencing genes that are already turned off must be considered.[35,36] As such, we cannot exclude the possibility that LDHA is silenced by an earlier mechanism and then methylated.

LDHA is essential for glycolysis and is overexpressed in cancers, especially those that are highly aggressive and treatment resistant. The IDH^{mt}-dependent silencing of *LDHA*, and perhaps other glycolytic genes as well, in conjunction with global downregulation of the HIF1α pathway through 2-HG–dependent promotion of HIF1α degradation[16] strongly suggests that IDH^{mt} gliomas may have limited glycolytic capacity. Moreover, a third mechanism may contribute to silencing of glycolytic genes in these cancers: upregulation of *SLC2A1*, *PDK1*, and *LDHA* by HIF1α requires the α-ketoglutarate–dependent histone demethylase Jumonji domain 2C,[37] which is potentially inhibited by 2-HG.[12] Thus, these 3 distinct mechanisms, all triggered by IDH^{mt} enzyme, may act in concert to

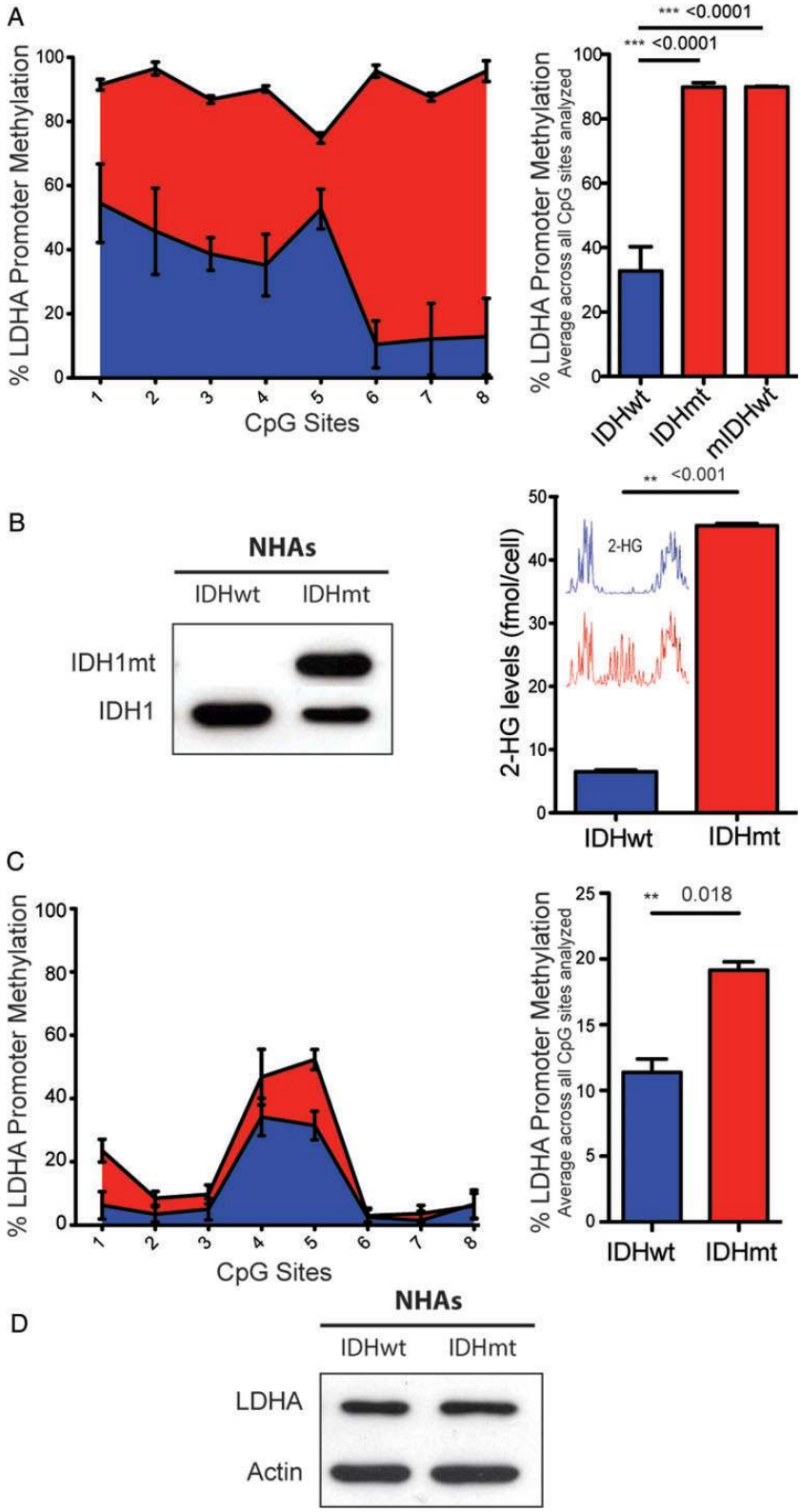

Fig. 5. IDH mutation correlates with *LDHA* promoter methylation. (A) Cytosine–phosphate–guanine (CpG) island methylation of the *LDHA* promoter in IDH^{wt} derived BTSCs (blue) and IDH^{mt} derived BTSCs (red) and average methylation of the region analyzed (bar graph). (B) Western blot showing the expression of mutant IDH1 and 2-HG levels in IDH^{mt} and IDH^{wt} NHAs reported in fmol/cell. (Insert: ^{1}H MR spectra showing the $H_{4,4'}$ resonance of 2-HG.) (C) CpG island methylation of the *LDHA* promoter in IDH^{mt} and IDH^{wt} NHAs, average methylation of the region analyzed (bar graph), and (D) Western blot showing *LDHA* expression in IDH^{mt} and IDH^{wt} NHAs.

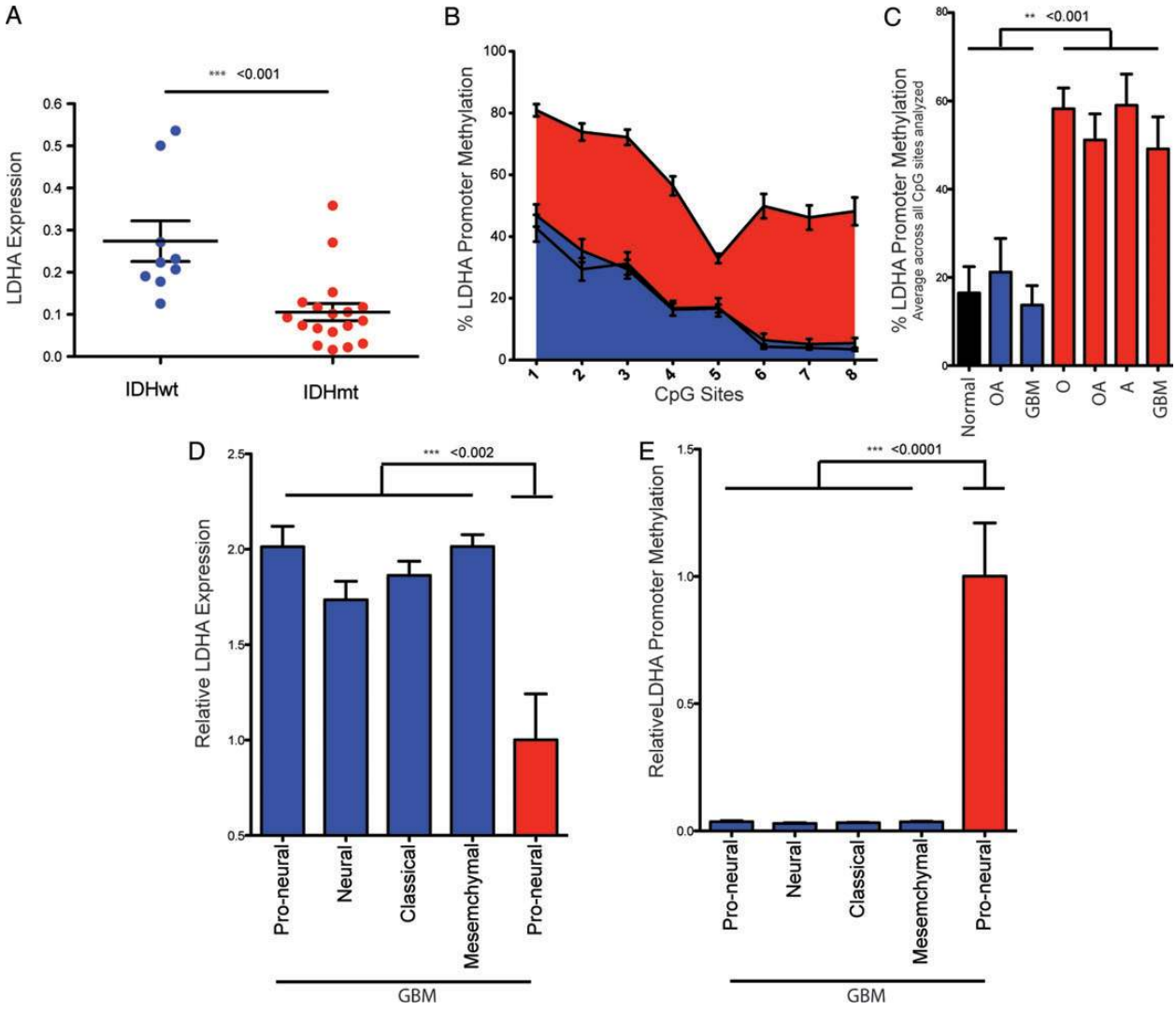

Fig. 6. *LDHA* silencing correlates with promoter methylation in IDH^{mt} glioma tissues. (A) *LDHA* expression in glioma tissue samples by RT-qPCR (blue: IDH^{wt}, red: IDH^{mt}). (B) CpG island methylation in *LDHA* promoter in normal brain tissue (black), IDH^{wt} (blue), or IDH^{mt} (red). (C) Average *LDHA* promoter methylation across all CpG sites analyzed in different types of gliomas subclassified as IDH^{wt} or IDH^{mt} (black: normal brain tissue; blue: IDH^{wt}; red: IDH^{mt}). (D) Relative *LDHA* expression and promoter methylation (E) in GBM from the TCGA database (Illumina HumanMethylation27, cg01316516) according to gene expression subtypes and IDH mutation (blue: IDH^{wt}, red: IDH^{mt}; values were normalized relative to IDH^{mt} proneural).

suppress glycolytic genes, suggesting that IDH^{mt} gliomas may have selected defects in glycolysis that could be of importance during early stages of tumorigenesis.

Isocitrate dehydrogenase mutant brain cancers do not behave like glycolytic tumors. Unlike primary glioblastomas and IDH^{wt} astrocytic gliomas, which are fast-growing, treatment-resistant cancers, gliomas harboring IDH mutations grow slowly, have a relatively good prognosis, and, in the case of oligodendrogliomas with 1p/19q codeletion, the prototypical IDH mutant cancer, display both enhanced radio- and chemosensitivity.[38] Consistent with this hypothesis are PET scan studies showing that codeleted oligodendrogliomas have a low affinity for ^{18}F-fluorodeoxyglucose, pointing to a metabolic profile that is not highly glycolytic. In keeping with these findings, our group has shown recently that the Na/H exchanger 1 gene (*NHE-1*) is silenced in oligodendrogliomas due to the combined effects of IDH^{mt}-dependent promoter methylation and 1p deletion.[39] As a result of NHE-1 loss, oligodendroglioma cells are unable to recover from an acid load, as would be generated by active glycolysis. Others had shown previously that only nonglycolytic cells are able to survive in the absence of NHE-1.[40] Interestingly, other genes critical for glycolysis, such as the glucose transporter *SLC2A1* and the lactate transporter *SLC16A1*, are also located on 1p and involved in the 1p/19q codeletion.

Whether IDH mutations through downregulation of glycolytic genes impact glioma metabolism, glycolytic activity, and tumor progression will require further investigation. However, based on results and observations, we hypothesize that IDH1/2 mutations, by downregulating essential glycolytic genes such as *LDHA*, may prevent the glycolytic switch and limit the rapid growth typical of high-grade gliomas.

To our knowledge, this is the first report of *LDHA* downregulation in a human cancer, typically a highly glycolytic disease.

Downregulation of glycolytic genes in IDH^{mt} gliomas is a surprising finding, but this unique feature may help to explain the slow disease progression of this subgroup of gliomas. This feature of IDH^{mt} gliomas may directly affect their behavior and, importantly, will provide further insights when designing targeted therapeutics to control IDH^{mt} human gliomas.

Supplementary Material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

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