In silico gene expression analysis reveals glycolysis and acetate anaplerosis in IDH1 wild-type glioma and lactate and glutamate anaplerosis in IDH1-mutated glioma

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ABSTRACT

Hotspot mutations in isocitrate dehydrogenase 1 (IDH1) initiate low-grade glioma and secondary glioblastoma and induce a neomorphic activity that converts α-ketoglutarate (α-KG) to the oncometabolite D-2-hydroxyglutarate (D-2-HG). It causes metabolic rewiring that is not fully understood. We investigated the effects of IDH1 mutations (IDH1MUT) on expression of genes that encode for metabolic enzymes by data mining The Cancer Genome Atlas. We analyzed 112 IDH1 wild-type (IDH1WT) versus 399 IDH1MUT low-grade glioma and 157 IDH1WT versus 9 IDH1MUT glioblastoma samples. In both glioma types, IDH1WT was associated with high expression levels of genes encoding enzymes that are involved in glycolysis and acetate anaplerosis, whereas IDH1MUT glioma overexpress genes encoding enzymes that are involved in the oxidative tricarboxylic acid (TCA) cycle. In vitro, we observed that IDH1MUT cancer cells have a higher basal respiration compared to IDH1WT cancer cells and inhibition of the IDH1MUT shifts the metabolism by decreasing oxygen consumption and increasing glycolysis. Our findings indicate that IDH1WT glioma have a typical Warburg phenotype whereas in IDH1MUT glioma the TCA cycle, rather than glycolytic lactate production, is the predominant metabolic pathway. Our data further suggest that the TCA in IDH1MUT glioma is driven by lactate and glutamate anaplerosis to facilitate production of α-KG, and ultimately D-2-HG. This metabolic rewiring may be a basis for novel therapies for IDH1MUT and IDH1WT glioma.

INTRODUCTION

Diffuse gliomas are infiltrative neoplasms that arise in the cerebral hemispheres of adults and are graded as WHO II-IV based on histopathological characteristics. Grade II and III are low-grade gliomas (LGG) of which a subset may progress to secondary glioblastoma (WHO grade IV), whereas others may remain relatively stable for longer periods of time. In contrast to this gradual progression of malignancy, the majority of glioblastomas arise de novo, and are then called primary glioblastoma. Complete surgical resection of diffuse glioma is impossible due to their highly invasive nature and without exception residual tumor is a source of recurrence and malignant progression. Median overall survival of LGG ranges widely from 3 to 15 years and patients with de novo primary glioblastoma, the most common and aggressive primary brain tumor, have a median overall survival of 16 months. Despite all efforts to therapeutically target various pathways that are involved in glioma progression, significant improvement in survival has not been achieved since 2005, when temozolomide was added to irradiation as standard therapy [1, 2].

A subset of gliomas displays increased sensitivity towards chemo- and radiotherapy with prolonged median overall patient survival. Recently, it was demonstrated that these characteristics are strongly associated with mutations in the metabolic enzyme isocitrate dehydrogenase (IDH) [3]. Hotspot mutations in IDH1 and, less frequently, IDH2
occur in 80% of WHO grade II-III and secondary WHO grade IV gliomas and are ancestral events in the formation of these neoplasms [4, 5]. In addition to glioma, IDH1/2 mutations (IDH1/2MUT) occur in substantial percentages in various other tumor types, such as acute myeloid leukemia (20–40%), chondrosarcoma (60%), intrahepatic cholangiocarcinoma (20%) and melanoma (5–10%) [6].

IDH1 and IDH2 are homodimeric enzymes that reversibly convert isocitrate to α-ketoglutarate (α-KG) with concomitant reduction of NADP+ to NADPH in the cytoplasm and mitochondria, respectively. The mutations lead to a neomorphic activity of the enzyme that converts α-KG into the oncometabolite D-2-hydroxyglutarate (D-2-HG). D-2-HG competitively inhibits α-KG-dependent enzymes [7, 8], including histone demethylases and 5-methylcytosine hydroxylases that are essential for epigenetic regulation of gene expression, including that of metabolic genes [9–13]. IDH1/2MUT changes cellular metabolism via 4 mechanisms: 1) loss of wild-type IDH1/2 (IDH1/2WT) function that affects carbohydrate and NADP+/NADPH metabolism; 2) accumulation of D-2-HG that further restricts the activity of various enzymes such as α-ketoglutarate dehydrogenase (α-KGDH), succinate dehydrogenase (SDH) and complex IV of the mitochondrial electron transport chain, 3) epigenetic effects of D-2-HG on expression of genes involved in metabolism and 4) increased degradation of the hypoxia-response transcription factor HIF-1α, a major inducer of expression of genes involved in glycolysis [3, 6, 14, 15].

Reprogramming of cellular metabolism is one of the hallmarks of cancer [16]. It is generally assumed that malignant cells use pyruvate for lactate production instead of fluxing it into acetyl-CoA in the TCA cycle, even in the presence of oxygen [17]. This so-called Warburg effect is an accepted metabolic phenotype in cancer that allows rapid generation of biosynthetic intermediates at the expense of less efficient ATP production. As a result, cancer cells overexpress glucose transporters to compensate for their ATP demand via an abnormally high rate of glucose uptake. Presumably, the composition and availability of metabolites in the microenvironment, for example in the adult brain glucose, glutamate, glutamine and acetate, dictates which metabolites are the predominant carbon sources for cancer cells. These metabolites can all serve as precursors for macromolecules such as proteins, nucleotides and lipids [18, 19].

It has been reported that glioma cells have a higher rate of aerobic glycolysis than healthy brain tissue, and this is more pronounced in glioblastoma than in LGG [20, 21]. However, these observations may be biased because most analyses of glioblastoma tissue use resection material that likely contains angiogenic, hypoxic and necrotic regions. Metabolism in these areas may well differ from that in peripheral invasive regions that presumably do not experience hypoxia. Talasila et al. [22] reported recently that the centre the tumor centre of glioblastoma is particularly glycolytic, in line with the presence of perinecrotic hypoxia, and we previously observed similar metabolic phenotypes in preclinical orthopic xenograft models [23]. However, how metabolic pathways in glioma are affected by IDH1MUT is not completely clear.

In order to evaluate the metabolic rewiring associated with IDH1WT, we performed an in silico analysis of the transcripts that encode for metabolic enzymes and substrate transporters. These gene expression data were correlated with IDH1 mutational status in 677 LGG and glioblastoma samples derived from The Cancer Genome Atlas (TCGA). This study provides a comprehensive overview of the expression of key glycolytic, TCA cycle and glucose/lactate/glutamate/acetate anaplerosis enzymes in IDH1MUT glioma versus IDH1WT glioma.

RESULTS

We determined differences in expression levels of metabolic enzymes in IDH1WT versus IDH1MUT glioma to increase our understanding of the metabolism of these two clinically very distinct types of gliomas. We included 399 IDH1MUT and 112 IDH1WT LGG (WHO stage II: 49% and WHO stage III: 51%) and 157 IDH1WT and 9 IDH1MUT WHO stage IV glioblastoma samples. The ratios of IDH1-mutated to IDH1 wild-type tumors are in agreement with mutation frequencies from earlier reports of TCGA glioma data and other datasets. In line with previous reports [4, 24, 25], our dataset shows an approximately 3-fold prolonged survival of IDH1MUT LGG and glioblastoma patients compared to IDH1WT LGG and glioblastoma patients (Supplementary Figure 1).

Expression of GLUT3 and glucose-metabolizing enzymes is low in IDH1MUT compared to IDH1WT glioma

To investigate the first rate-limiting step of cellular glucose metabolism, i.e. transport of glucose across the plasma membrane, we examined expression levels of SLC2A1 and SLC2A3, the genes encoding for glucose transporters (GLUT1 and GLUT3). GLUT3 expression levels were low in IDH1MUT glioma when compared with IDH1WT glioma, whereas GLUT1 expression did not differ between IDH1MUT and IDH1WT glioma (Figure 1A). The rate-limiting, irreversible and glycolysis-related enzymes hexokinase (HK2 and HK3) and pyruvate kinase (PKM2), but not HK1 or the pyruvate kinase isofrom PKLR, were also expressed at lower levels in IDH1MUT glioma compared with IDH1WT glioma (Figure 1). High expression of HK2 was significant in IDH1WT LGG, whereas HK3 expression was significant higher in IDH1WT glioblastoma. The rate-limiting enzyme of the pentose-phosphate pathway, glucose-6-phosphate dehydrogenase (G6PD) was expressed at higher levels in IDH1WT glioma, but only in LGG and not glioblastoma. These results
suggest that IDH1\textsuperscript{WT} glioma depend more on glycolysis than IDH1\textsuperscript{MUT} glioma.

**IDH1\textsuperscript{WT} glioma express Warburg-effect genes, whereas IDH1\textsuperscript{MUT} glioma express TCA cycle genes**

The relative activities of lactate fermentation in the glycolysis and the TCA cycle are controlled by two sets of enzymes that determine the fate of pyruvate: pyruvate dehydrogenase (PDH) and the lactate dehydrogenases (LDHA and LDHB, where LDHA catalyzes conversion of pyruvate into lactate and LDHB catalyzes the reverse reaction). In IDH1\textsuperscript{WT} glioma, we observed a striking overexpression of LDHA relative to IDH1\textsuperscript{MUT} glioma (Figure 1A). Lower expression levels of LDHA in IDH1\textsuperscript{MUT} glioma were associated with hypermethylation of its promoter (Figure 1B). Conversely, IDH1\textsuperscript{MUT} glioma showed higher LDHB expression levels than IDH1\textsuperscript{WT} glioma. To maintain pH homeostasis, cancer cells up-regulate transporters such as the monocarboxylate transporters 1 and 4 (MCT1 and MCT4) for secretion of lactate [26, 27]. In IDH1\textsuperscript{WT} glioma, we observed higher expression of MCT1 and MCT4 as compared to IDH1\textsuperscript{MUT} glioma, suggesting increased transport of lactate in IDH1\textsuperscript{WT} glioma. These results indicate that IDH1\textsuperscript{WT} gliomas catabolize glucose in the glycolytic pathway with lactate as end product whereas in IDH1\textsuperscript{MUT} glioma lactate is converted into pyruvate.

Conversion of pyruvate into acetyl-CoA for its entry into the TCA is accomplished by the pyruvate dehydrogenase (PDH) complex, the activity of which is controlled by pyruvate dehydrogenase kinase (PDK; isoforms PDK1, PDK2 and PDK3). PDK inactivates PDH via phosphorylation. PDH is a heterotrimeric enzyme complex composed of subunits PDHA and PDHB, forming the catalytic PDH component PDH E1, and PDHX, a non-catalytic regulatory subunit. In IDH1\textsuperscript{WT} glioma, we observed higher expression of regulator genes PDK1 and PDK3 as compared to IDH1\textsuperscript{MUT} glioma. On the other hand, IDH1\textsuperscript{MUT} glioma showed higher expression levels of PDHA (in glioblastoma), PDHB (in LGG) and PDHX as compared to IDH1\textsuperscript{WT} glioma (Figure 2). Combined with the aforementioned glycolysis data these results suggest that in contrast to IDH1\textsuperscript{WT} glioma, IDH1\textsuperscript{MUT} glioma actively prevents the generation of lactate by silencing LDHA and maintaining high expression of LDHB, PDHA/B and PDHX. These conditions ensure that pyruvate is maximally used for TCA entry.

**IDH1\textsuperscript{MUT} glioma have increased expression of TCA genes upstream of isocitrate and decreased expression of TCA genes downstream of isocitrate**

Intermediate metabolites of glycolysis upstream of pyruvate can be shunted into macromolecular biosynthesis pathways without stalling glycolysis itself. In contrast, the TCA cycle must be continuously running and be completed to function optimally: generation of citrate by citrate synthase (CS) requires pyruvate-derived acetyl-CoA, the principal fuel of mitochondria, and the last TCA cycle metabolite (oxaloacetate). Mitochondrial products of a full TCA cycle are ATP and CO\textsubscript{2}. The lack of glycolysis in IDH1\textsuperscript{MUT} gliomas suggests that these cancers apply alternative metabolic pathways that ensure sufficient energy and building blocks for macromolecular biosynthesis. To investigate this in detail, we examined expression levels of TCA-related enzymes in IDH1\textsuperscript{MUT} and IDH1\textsuperscript{WT} gliomas.

CS and aconitase (ACO1/2), enzymes that function upstream of citrate oxidation, were expressed at higher levels in IDH1\textsuperscript{MUT} glioma than in IDH1\textsuperscript{WT} glioma (Figure 2). In contrast, expression levels of enzymes involved in downstream metabolism of isocitrate, including IDH1, IDH2 and IDH3A, α-KGDH, succinate dehydrogenase (SDHB), fumarate hydratase (FH) and malate dehydrogenase (MDH2), were remarkably lower in IDH1\textsuperscript{MUT} glioma versus IDH1\textsuperscript{WT} glioma. These results suggest that IDH1\textsuperscript{MUT} glioma have an imbalanced TCA, with increased enzyme activity upstream of isocitrate, and decreased TCA cycle metabolism downstream of isocitrate. The imbalance resulting from low oxaloacetate production and high pyruvate influx causes disbalance that may be problematic for efficient citrate production. As a compensatory mechanism, oxaloacetate can also be produced directly from pyruvate by the activity of pyruvate carboxylase (PC). This reaction is counteracted by phosphoenolpyruvate carboxykinase 2 (PCK2) which catalyzes the reaction of oxaloacetate to phosphoenolpyruvate. In IDH1\textsuperscript{MUT} glioma, PC levels were markedly higher than in IDH1\textsuperscript{WT} glioma, whereas PCK2 expression levels were significantly lower in IDH1\textsuperscript{MUT} glioma although differences in PCK2 levels between IDH1\textsuperscript{WT} and IDH1\textsuperscript{MUT} in glioblastoma were less apparent (Figure 3). Collectively, these results indicate increased flux of pyruvate into the TCA cycle in IDH1\textsuperscript{MUT} glioma compared to IDH1\textsuperscript{WT} counterparts.

**Higher lactate/glutamate anaplerosis in IDH1\textsuperscript{MUT} glioma and higher acetate anaplerosis in IDH1\textsuperscript{WT} glioma**

We have previously postulated the hypothesis that glutamate could act as anaplerotic precursor for the TCA cycle in IDH1\textsuperscript{MUT} glioma [28]. To test this assumption, we analyzed the expression levels of mRNA of enzymes that are involved in the transport and conversion of these metabolites.

Glutamine is a major source of TCA cycle metabolites via glutaminolysis, a process that converts glutamine to α-KG via two subsequent deamination steps. To investigate its contribution to α-KG replenishment in the TCA cycle, we determined mRNA expression levels of glutaminase (GLS and GLS2, converting glutamine to glutamate) and...
glutamate dehydrogenase (GLUD1 and GLUD2, converting glutamate to α-KG). We also investigated mRNA expression levels of branched-chain amino acid transferase (BCAT1 and BCAT2) and glutamine synthetase (GS), which catalyze the converse reaction of α-KG into glutamate and of glutamate into glutamine, respectively. In $\text{IDH}^{\text{MUT}}$ glioma, we observed strikingly higher expression levels of GLUD1/2 than in $\text{IDH}^{\text{WT}}$ glioma, whereas BCAT1/2 expression levels were much lower in $\text{IDH}^{\text{MUT}}$ gliomas than in $\text{IDH}^{\text{WT}}$ glioma (Figure 3), as has been reported.

Figure 1: mRNA expression levels of enzymes involved in glucose metabolism in $\text{IDH}^{\text{WT}}$ versus $\text{IDH}^{\text{MUT}}$ glioma. $\text{IDH}^{\text{WT}}$ glioma show higher expression levels of genes that are typically upregulated in glycolytic cancer cells. (A) Analysis of gene expression levels of metabolic enzymes involved in transport and metabolism of glucose in $\text{IDH}^{\text{WT}}$ ($n = 399$) and $\text{IDH}^{\text{MUT}}$ ($n = 112$) LGG and $\text{IDH}^{\text{WT}}$ ($n = 157$) and $\text{IDH}^{\text{MUT}}$ ($n = 9$) glioblastoma samples obtained from the TCGA database. Relative mRNA expression levels are shown for $\text{IDH}^{\text{WT}}$ (blue) and $\text{IDH}^{\text{MUT}}$ (red). (B) $\text{LDHA}$ and (C) $\text{LDHB}$ expression and methylation according to $\text{IDH}^{\text{MUT}}$ status (blue: $\text{IDH}^{\text{WT}}$, red: $\text{IDH}^{\text{MUT}}$). Abbreviations: GLUT, glucose transporter; HK, hexokinase; G6PD, glucose-6-phosphate dehydrogenase; PK, pyruvate kinase; LDH, lactate dehydrogenase.
Previously [29], low BCAT1 mRNA expression levels in \textit{IDH1}^{MUT} glioma were associated with BCAT1 promoter hypermethylation (Figure 3B).

We observed no significant differences between mRNA expression levels of GLS, GLS2, or GS between \textit{IDH1}^{WT} glioma and \textit{IDH1}^{MUT} glioma. These results suggest that \textit{IDH1}^{MUT} gliomas predominantly utilize glutamate, rather than glutamine, for TCA cycle anaplerosis.

Acetate can be utilized as an alternative substrate to generate acetyl-CoA and is transferred through the plasma membrane by MCT1 and MCT4 [30]. Acetyl-CoA synthetase (ACSS1 and ACSS2) converts acetate to acetyl-CoA, which can be catabolized in the TCA cycle. In \textit{IDH1}^{WT} glioma, ACSS2 showed an elevated mRNA expression compared to \textit{IDH1}^{MUT} glioma (Figure 3), suggesting a higher contribution of acetate to the acetyl-CoA pool in \textit{IDH1}^{WT} glioma. As indicated previously, higher contribution of acetate is in line with higher expression levels of MCT1 and MCT4 transporters in \textit{IDH1}^{WT} glioma.

Taken together, we show that relative to \textit{IDH1}^{WT} glioma, \textit{IDH1}^{MUT} glioma use lactate and glutamate (but lesser so glutamine) for the TCA cycle, by maintaining high expression of LDHB, PC, and GLUD and silencing BCAT. In contrast, in \textit{IDH1}^{WT} glioma glycolysis is more prominent and acetate is used to fuel the TCA cycle.

**Inhibition of the \textit{IDH1}^{MUT} decreases oxygen consumption and increases lactate acidification**

There is a scarcity of relevant models to study metabolic effects of IDH mutations \textit{in vitro} [31]. As an alternative we used the HCT116-IDH1R132H knock in cell line and its parental counterpart to investigate whether metabolic

![Figure 2: Expression levels of genes of enzymes involved in the TCA cycle in \textit{IDH1}^{WT} versus \textit{IDH1}^{MUT} glioma. Increased TCA upstream of isocitrate, but decreased metabolism downstream of isocitrate in \textit{IDH1}^{MUT} glioma as indicated by gene expression levels of enzymes involved in the TCA cycle in \textit{IDH1}^{WT} (n = 399) and \textit{IDH1}^{MUT} (n = 112) LGG and \textit{IDH1}^{WT} (n = 157) and \textit{IDH1}^{MUT} (n = 9) glioblastoma. Relative mRNA expression levels are shown for \textit{IDH1}^{WT} (blue) and \textit{IDH1}^{MUT} (red). Abbreviations: PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; CS, citrate synthase; ACO, aconitase; IDH, isocitrate dehydrogenase; a-KGDH, \(\alpha\)-ketoglutarate dehydrogenase; SDH, succinate dehydrogenase; FH, fumarate hydratase; MDH, malate dehydrogenase.](https://www.impactjournals.com/oncotarget/49169)
changes in \( IDH_1^{R132H} \) gliomas were reflected in this isogenic cell line pair too. We first measured oxygen consumption in HCT116 \( IDH_1^{WT/R132H} \) and HCT116 \( IDH_1^{WT/WT} \) cells in the Seahorse\[32\]. The basal respiration was significantly higher in HCT116 \( IDH_1^{WT/R132H} \) cells than in HCT116 \( IDH_1^{WT/WT} \) cells, showing that also in this system \( IDH_1^{MUT} \) cells have higher oxygen consumption and are more dependent on OXPHOS (Figure 4). To determine whether the \( IDH_1^{MUT} \) is causally involved in the higher respiration rates, we tested the effects of the specific \( IDH_1^{R132H} \) inhibitor AGI-5198. In line with expectations after 14 days of culture in the presence of the \( IDH_1^{MUT} \) inhibitor AGI-5198 the respiration rate of HCT116 \( IDH_1^{WT/132H} \) cells was significantly decreased and comparable to levels of HCT116 \( IDH_1^{WT/WT} \) cells (Figure 4B).

In order to investigate the glycolytic activity, we measured the extracellular acidification rate (ECAR) that determines the glycolytic phenotype of cells and is linearly related to lactate production\[33, 34\]. In HCT116 \( IDH_1^{WT/R132H} \) cells, we found a lower ECAR response to glucose compared to HCT116 \( IDH_1^{WT/WT} \) cells, indicating a lower glycolytic rate in \( IDH_1^{MUT} \) cells (Figure 4). The glycolytic capacity was also significantly lower in HCT116 \( IDH_1^{WT/R132H} \) cells compared to HCT116 \( IDH_1^{WT/WT} \) cells (data not shown). After 14 days incubation in the presence of AGI-5198, we measured a slight increase of ECAR in HCT116 \( IDH_1^{WT/R132H} \) cells, indicating a shift of metabolism to glycolysis by inhibiting \( IDH_1^{MUT} \) (Figure 4C). Taken together, we show that relative to \( IDH_1^{WT} \) cells, \( IDH_1^{MUT} \) cells are dependent on OXPHOS and inhibition of the \( IDH_1^{MUT} \) decreases the oxygen consumption leading to a glycolytic phenotype.

**DISCUSSION**

The present study is the first to investigate the metabolic rewiring that is associated with \( IDH_1^{MUT} \) in glioma in a comprehensive and integrated fashion. A summary of this metabolic rewiring is shown in Figure 5. We correlated the expression of key glycolytic, TCA cycle and glucose/lactate/glutamate/anaplerosis enzymes with the \( IDH_1^{MUT} \) status obtained from a large database containing data on both LGG and glioblastoma patients. We show that \( IDH_1^{WT} \) glioma overexpress enzymes that play key roles in anaerobic glycolysis, i.e. genes that are typically upregulated in Warburg-like cancer cells. In

![Figure 3: Contribution of glucose/glutamine/glutamate anaplerosis and acetate influx to TCA cycle metabolism in \( IDH_1^{WT} \) versus \( IDH_1^{MUT} \) glioma.](image-url)

**Figure 3:** Contribution of glucose/glutamine/glutamate anaplerosis and acetate influx to TCA cycle metabolism in \( IDH_1^{WT} \) versus \( IDH_1^{MUT} \) glioma. Increased glucose/glutamate anaplerosis in \( IDH_1^{MUT} \) glioma and high contribution of acetate in \( IDH_1^{WT} \) glioma metabolism. (A) Gene expression levels of metabolic enzymes involved in glucose anaplerosis, glutaminolysis and acetate metabolism in \( IDH_1^{WT} \) \((n=399)\) and \( IDH_1^{MUT} \) \((n=112)\) LGG and \( IDH_1^{WT} \) \((n=157)\) and \( IDH_1^{MUT} \) \((n=9)\) glioblastoma. Relative mRNA expression levels are shown for \( IDH_1^{WT} \) (blue) and \( IDH_1^{MUT} \) (red). (B) \( BCAT1 \) expression and methylation according to \( IDH_1^{MUT} \) status (blue: \( IDH_1^{WT} \), red: \( IDH_1^{MUT} \)). Abbreviations: PC, pyruvate carboxylase; GLUD, glutamate dehydrogenase; GLS, glutaminase; BCAT, branched-chain amino acid transferase; GS, glutamine synthetase; ACSS, acetyl-CoA synthetase.
In contrast, expression of genes that encode for glycolytic metabolism are downregulated in IDH1MUT glioma. Based on the axiom that gene expression levels are correlated with pathway activity, our data suggest that IDH1MUT glioma predominantly catabolize glutamate, and to a lesser extent glucose, in the TCA cycle for α-KG production. In support of this axiom, we confirmed these observations in functional studies by measuring lactate production and oxygen consumption in HCT116 IDH1WT/WT and HCT116 IDH1WT/R132H cells.

Glycolysis and lactate production are considered to be the major metabolic ways of processing of glucose in cancer cells as it generates both energy and carbon for macromolecular biosynthesis, required for sustained cell proliferation [35]. In particular, GLUT3, HK2, PKM2 and LDHA play critical roles in initiating and maintaining the high glycolytic rates of rapidly proliferating cancer cells, and are associated with greater dependence on glycolysis than on OXPHOS [36–40]. Our findings are in agreement with the existence of the Warburg effect in IDH1WT glioma, whereas our data indicate that glycolysis and lactate production occur to a lesser extent in IDH1MUT glioma. In line with this notion, silencing of HK2 increases oxygen consumption and decreases lactate production in glioblastoma cells [41]. The elevated levels of LDHB in IDH1MUT gliomas support the findings in previous studies that showed that IDH1MUT glioma have reduced intracellular lactate levels compared to IDH1WT glioma, [42, 43]. Moreover, a recent study showed that the conversion rate of pyruvate into lactate was similar in IDH1MUT glioma and normal brain, whereas IDH1WT glioma showed a higher lactate production than normal brain [44]. Our results also support previous reports that show that IDH1MUT gliomas silence LDHA expression through hypermethylation of its promoter [43] resulting in a low LDHA/LDHB ratio compared with IDH1WT glioma or normal brain tissue [45]. Reduced MCT expression in IDH1MUT glioma is consistent with reduced glycolytic production of lactate as well as silencing of LDHA expression; cells that produce less lactate do not require an increase in its export and are less dependent on MCT expression.

Lactate production by IDH1WT glioma cells has been suggested to acidify the extracellular space [46, 47] resulting in the death of surrounding normal brain cells and increasing the infiltrative potential of cancer cells [48]. Our results suggest that IDH1MUT glioma produce and secrete less lactate than IDH1WT glioma which may correlate with a normalized tissue pH in IDH1MUT glioma and the less aggressive behavior of IDH1MUT glioma as compared to IDH1WT glioma [49].

Compared with IDH1WT glioma, IDH1MUT glioma showed elevated expression levels of TCA cycle genes that function upstream of isocitrate whereas expression of TCA cycle genes downstream of isocitrate were decreased. In

![Figure 4: The metabolic phenotype of IDH1MUT and IDH1WT.](image-url)
In line with these observations, a recent study reported high expression of CS and ACO2 in IDH1\(^{WT}\) glioma versus IDH1\(^{MUT}\) glioma [45]. We have shown previously that IDH enzymatic activity in IDH1\(^{MUT}\) glioblastoma is reduced compared to IDH1\(^{WT}\) glioblastoma [25]. Consistent with these findings, a previous report demonstrated increased levels of isocitrate (2.7-fold) and D-2-HG (28-fold) in IDH1\(^{MUT}\) cancer cells versus IDH1\(^{WT}\) cancer cells [8, 50]. In addition to decreased SDH expression in IDH1\(^{MUT}\) glioma, a recent study demonstrated elevated succinate levels and protein succinylation in IDH1\(^{MUT}\) glioma samples [51]. Taken together, the changes in gene expression levels in IDH1\(^{MUT}\) suggest that mitochondrial isocitrate production is maximized, providing substrate for the cytosolic IDH1\(^{WT}/IDH1^{MUT}\) dimer complexes to generate α-KG and D-2-HG, respectively.

Due to the lack of stable glioma cell cultures carrying an endogenous IDH1\(^{R132H}\) allele, we used in vitro HCT116 colorectal carcinoma cells for functional studies. IDH1\(^{MUT}\) occur in 0.5% of colorectal carcinomas [52]. Because IDH1\(^{R132H}\) functions as a heterodimer with IDH1\(^{WT}\), 1:1 IDH1\(^{R132H}/IDH1^{WT}\) expression in IDH1\(^{WT}/R132H\) HCT116 cells is more relevant than IDH1\(^{R132H}\) overexpression models that are frequently used. Previous reports have shown that HCT116 IDH1\(^{MUT}\) cells have reduced growth rates as compared to IDH1\(^{WT}\) cells under conditions of low oxygen tension suggesting that these are more dependent on OXPHOS [53].

Another major contributor to TCA metabolism in cancer cells is glutamine, which provides α-KG via the glutaminolysis pathway. Moreover, 80% of D-2-HG generated by heterozygous IDH1\(^{WT/MUT}\) HCT116 colorectal cancer cells is derived from glutamine [53, 54], rendering this an important metabolite for D-2-HG generation. Whereas glutamine has been suggested to be an important carbon source in several types of cancers, experiments in xenograft models revealed that glioblastoma cells take up glutamine but do not directly oxidize it in the TCA cycle [55]. Our analysis suggests an increased contribution of glutamate, rather than glutamine, to the TCA cycle in IDH1\(^{MUT}\) glioma, by maintaining high expression levels of GLUD1/2 and silencing or downregulation of BCAT expression. Our results support the findings of previous studies that demonstrated increased conversion of glutamate to α-KG by GLUD [45, 56] and reduced conversion of α-KG to glutamate by BCAT1 in IDH1\(^{MUT}\) glioma, which is in line with promoter methylation of BCAT1 in IDH1\(^{MUT}\) glioma [29, 57].

In line with our observations, a recent study demonstrated that IDH1\(^{MUT}\) overexpression in astrocytes results in increased PC expression and increased fractional flux through PC, suggesting that direct oxaloacetate production from pyruvate is critical for IDH1\(^{MUT}\) cancer cells to maintain TCA activity [58]. Furthermore, metabolic flux studies in IDH1\(^{MUT}\) glioma models with [1–\(^{13}\)C]-glucose showed that 20% of 2-HG was derived from glucose, suggesting that glucose is a less relevant donor of carbon for 2-HG production than glutamate [54]. On the other hand, we observed a high expression of PCK2 that shuttles oxaloacetate into glycolysis suggesting increased contribution from the TCA cycle to glycolysis in IDH1\(^{WT}\) glioma. In line with this observation, a study

**Figure 5: Rewiring of metabolism by IDH1\(^{MUT}\).** IDH1\(^{WT}/IDH1^{MUT}\) and IDH1\(^{MUT}/IDH1^{WT}\) ratios indicate contribution of a particular pathway as calculated on the basis of gene expression levels in IDH1\(^{WT}\) and IDH1\(^{MUT}\), respectively. IDH1\(^{WT}\) glioma catabolize glucose in the glycolysis with lactate as end product, whereas IDH1\(^{MUT}\) glioma prefers production of pyruvate from lactate and use the TCA cycle to generate isocitrate. Glucose, lactate and glutamate replenish the TCA cycle in IDH1\(^{MUT}\) glioma, to facilitate α-KG production for consumption by IDH1\(^{MUT}\) to generate 2-HG, whereas acetate anaplerosis is important in IDH1\(^{WT}\) glioma.
also reported high expression of PCK2 in IDH1\textsuperscript{WT} glioma versus IDH1\textsuperscript{MUT} glioma [45].

Finally, acetate metabolism can replenish the TCA cycle at the level of acetyl-CoA and this is an important anaplerosis pathway in the majority of glioblastoma [55]. Our analyses suggest that this phenomenon is more important in IDH1\textsuperscript{WT} glioma than in IDH1\textsuperscript{MUT} glioma. Mashimo et al. did not stratify their glioblastoma cohort based on IDH1\textsuperscript{MUT} status but demonstrated higher expression of ACSS2 in glioblastoma than in grade II and III glioma and high ACSS2 expression in LGG patients with poor survival as opposed to low ACSS2 expression in LGG patients with prolonged survival. This resonates with the high frequency of IDH1\textsuperscript{MUT} in LGG cohorts and clarifies the observed association of ACSS2 expression and LGG patient survival, potentially reflecting the differences in ACSS2 expression and patient survival between IDH1\textsuperscript{MUT} and IDH1\textsuperscript{WT} glioma.

Our transcriptome-wide approach study was performed on a large number of LGG and glioblastoma samples derived from TCGA. These data have been analyzed previously in an effort to improve the pathological classification of glioma. It was proposed to categorize LGG in 3 molecular classes, based on IDH1, 1p/19q and TP53 status, instead of histology [59]. In addition, IDH1\textsuperscript{WT} LGG appeared to be molecularly and clinically more similar to IDH1\textsuperscript{WT} glioblastoma than to IDH1\textsuperscript{MUT} LGG. Our studies support these conclusions in more detail, based on the profound similarities in metabolic transcript profiles both in IDH1\textsuperscript{WT} gliomas and in IDH1\textsuperscript{MUT} gliomas, irrespective of WHO grade. Of note, our data are confirmed by independent studies showing lower activity of HK, SDH and ACSS2 and higher activity of CS in LGG compared to glioblastoma [41, 55, 60]. Considering the fact that IDH1\textsuperscript{MUT} is more prevalent in LGG than in glioblastoma (80% versus 12%), this suggests that IDH1\textsuperscript{MUT} is an important determinator in histological analyses and that metabolic analyses should be stratified by IDH1\textsuperscript{MUT} status and not histology.

These insights are relevant for rational development of metabolic anti-glioma therapies, because our data predict that inhibitors of glycolysis, such as HK2/3 and PKM2 [61, 62] may be the most promising for IDH1\textsuperscript{WT} glioma, whereas inhibitors of the TCA cycle and the glutamatolysis pathway such as metformin, chloroquine and epigallocatechin-3-gallate [3, 56] may possess a therapeutic index in IDH1\textsuperscript{MUT} glioma.

**MATERIALS AND METHODS**

**Patient selection**

Gene expression analysis of LGG and glioblastoma data from TCGA (http://cancergenome.nih.gov) was conducted on processed data of the UCSC Cancer Genome Browser portal [63] and the eBioPortal for Cancer Genomics [64, 65]. The molecular data of 399 IDH1\textsuperscript{MUT} versus 112 IDH1\textsuperscript{WT} LGG and 157 IDH1\textsuperscript{WT} versus 9 IDH1\textsuperscript{MUT} glioblastoma samples were subsetted according to the IDH1 mutational status and analyzed for expression and methylation of metabolic enzymes. Another 16 LGG and 198 glioblastoma tumors were excluded because of unknown IDH1 mutational status or lack of molecular data.

**In silico analysis**

Relative mRNA expression (RNAseq, IlluminaHiSeq) values were identified for relevant genes encoding for metabolic enzymes involved in glycolysis, the TCA cycle, glutamine-glutamate cycle and acetate cycle (Supplementary Table 1). Annotations were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG). Mean normalized z-scores for mRNA levels were determined for IDH1\textsuperscript{MUT} and IDH1\textsuperscript{WT} datasets. The expression of each gene in each sample was normalized separately. The returned value indicated the number of standard deviations away from the population mean. In the methylation data, the probes with the strongest negative correlation between the methylation signal and gene expression were included.

**Cell culture**

HCT116 IDH1\textsuperscript{WT/R132H} knock-in cells, generated by AAV targeting technology GENESIS (28), were kindly provided by Horizon Discovery (Cambridge, UK). IDH1\textsuperscript{WT/R132H} and IDH1\textsuperscript{WT/WT} HCT116 cells were cultured in McCoy’s 5A medium (Gibco, Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA) in 5% CO\textsubscript{2} at 37°C. Media was supplemented with 10% fetal bovine serum (HyClone, Thermo Fisher Scientific), 100 units/ml penicillin and 100 μg/ml streptomycin (both Gibco).

**Reagents**

AGI-5198 was purchased from MedChemExpress (Monmouth Junction, NJ, USA), olygomycin, carbonyl-cyanide-(trifluoromethoxy)phenylhydrazone (FCCP), antimycin A, rotenone, 2-deoxyglucose, glucose, L-glutamine and sodium pyruvate were purchased from Sigma-Aldrich. (St. Louis, MO, USA).

**Measurement of oxygen consumption rate and extracellular acidification rates**

HCT 116 cells, with and without pre-treatment of 1μM AGI-5198, were grown in 5% CO\textsubscript{2} at 37°C and OCR and ECAR was measured using an XFe96 analyzer (Seahorse Bioscience, North Billerica, MA, USA). HCT116 cells were plated in XF96 cell culture plates, 2.0*10\textsuperscript{4} IDH1\textsuperscript{WT/R132H} HCT116 cells or 1.75*10\textsuperscript{4} IDH1\textsuperscript{WT/WT} cells (to account for a more rapid proliferation of IDH1\textsuperscript{WT/WT} HCT116 cells relative to IDH1\textsuperscript{WT/R132H}}
HCT116 cells ((53) and our own observation), were seeded in each well of 96-well assay plates and incubated for 48 h prior to conducting the assay. For determination of OCR, medium was changed to DMEM supplemented with 10 mM glucose, 2 mM glutamine and 1.5 mM pyruvate. For the glycolytic flux, medium was changed to DMEM supplemented with 2 mM L-glutamine and the concentration of glucose added to initiate glycolysis was 25 mM. Four independent replicates were conducted with 10 technical replicates per cell line. Data were expressed as pmol of O$_2$ per minute and normalized by cell number measured by the CyQUANT Cell proliferation kit (Invitrogen™), which is based on a fluorochrome binding to nucleic acids. Fluorescence was measured in a microplate luminometer (ClarioStar BMG Labtech, Cary, NC, USA) with excitation wavelength at 485 ± 10 nm and emission detection wavelength at 530 ± 12.5 nm.

Statistical analysis

Statistical analysis included two-way Mann-Whitney tests to determine significance of expression differences observed between IDH1$^{MUT}$ versus IDH1$^{WT}$ samples and Kaplan–Meier estimates of survival with log-rank tests among strata. Relative expression data were visualized as box plots, depicting the 25th, 50th and 75th percentile, with 95% confidence intervals and significance levels are shown by (*$P < 0.05$), (**$P < 0.01$), (***$P < 0.001$) and (****$P < 0.0001$).

CONFLICTS OF INTEREST

The authors declare no competing interests.

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