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Magnetic Resonance Spectroscopy of *IDH*-mutated gliomas: current knowledge on the neurochemical profile

Francesca Branzoli, PhD^{1,2}, Małgorzata Marjańska, PhD³

¹Institut du Cerveau – ICM, Centre de Neuroimagerie de Recherche – CENIR, Paris, France

²ICM, INSERM U 1127, CNRS UMR 7225, Sorbonne Université, Paris, France

³Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota, Minneapolis, MN, USA

Abstract

Purpose of review: Magnetic resonance spectroscopy (MRS) may play a key role for the management of patients with glioma. We highlighted the utility of MRS in the noninvasive diagnosis of gliomas with mutations in isocitrate dehydrogenase (*IDH*) genes, by providing an overview of the neurochemical alterations observed in different glioma subtypes, as well as during treatment and progression, both in vivo and ex vivo.

Recent findings: 2-hydroxyglutarate (2HG) decrease during anti-cancer treatments was recently shown to be associated with altered levels of other metabolites, including lactate, glutamate and glutathione, suggesting that tumor treatment leads to a metabolic reprogramming beyond 2HG depletion. In combination with 2HG quantification, cystathionine and glycine seem to be the most promising candidates for higher specific identification of glioma sub-types and follow-up of disease progression and response to treatment.

Summary: The implementation of advanced MRS methods in the routine clinical practice will allow the quantification of metabolites that are not detectable with conventional methods and may enable immediate, accurate diagnosis of gliomas, which is crucial for planning optimal therapeutic strategies and follow-up examinations. The role of different metabolites as predictors of patient outcome still needs to be elucidated.

Keywords

IDH mutation; glioma; brain metabolites; treatment effect; progression

Introduction

In 2009, mutations of the genes encoding the enzymes isocitrate dehydrogenase 1 and 2 (*IDH1/2*) were discovered in gliomas [1]. *IDH* mutations are found in ~90% of diffuse grade II and 60% of diffuse grade III gliomas, 5% of primary glioblastomas (GBM), and are

Corresponding author: Francesca Branzoli, Ph.D., Institut du cerveau - ICM, Hôpital Pitié-Salpêtrière, 47 boulevard de l'Hôpital, CS 21414, 75646 Paris Cedex 13, Phone number: +33 (0)1 57 27 46 46, francesca.branzoli@icm-institute.org.

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absent in other brain tumors [2]. *IDH*-mutated gliomas form a biologically independent subset of tumors associated with a 2- to 4-fold longer median survival compared to wild-type (WT) tumors [3]. Owing to its high diagnostic and prognostic value, *IDH* mutational status was integrated into the 2016 World Health Organization classification of gliomas [4]. Diffuse glioma classification requires histologic evaluation and assessment of the *IDH* mutational status and codeletion status of chromosomal arms 1p and 19q (1p/19q codeletion) [5]. 1p/19q codeletion is found only in *IDH*-mutated gliomas, is linked with the oligodendroglial histologic subtype, and is associated with the best prognosis. *IDH*-mutated 1p/19q-non-codeleted gliomas are classified as diffuse astrocytomas and are associated with intermediate outcome, while *IDH*-WT have the poorest prognosis [2].

Magnetic resonance imaging (MRI) plays a vital role in the diagnosis and management of patients with brain tumors [6**]. Among different MRI modalities, in vivo ¹H MR spectroscopy (MRS) provides unique metabolic information through the measurement of the neurochemical concentrations in tissue.

The aim of this review is to provide an overview of the metabolic alterations observed in different glioma subtypes both in vivo and ex vivo, and to highlight the possible role of ¹H MRS in the noninvasive diagnosis of *IDH*-mutated gliomas, as well as in monitoring the effect of tumor progression and anti-cancer treatments on the neurochemical profile.

D-2-hydroxyglutarate

IDH mutations result in the overproduction of D-2-hydroxyglutarate (2HG) (Figure 1) and have been suggested to cause changes in global cellular metabolism, either in consequence of increased 2HG or through inhibition of isocitrate metabolism [7]. In vivo MRS enables the noninvasive detection of 2HG, potentially providing a great impact on patient management.

Clinical relevance

Noninvasive 2HG detection by MRS is a powerful tool for immediate, pre-surgical diagnosis of *IDH*-mutated gliomas. Indeed, 2HG MRS allows in first place to differentiate noninvasively gliomas from metastases, primary brain lymphomas or other non-neoplastic lesions. In vivo assessment of *IDH* status is highly beneficial for neurosurgical planning as maximal surgical resection has been shown to confer a better prognosis in *IDH*-mutated gliomas compared to WT [8]. Thus, although in vivo MRS is not meant to replace surgery or biopsy, which are needed for histological confirmation of the *IDH* status and assessment of the tumor genetic profile, pre-operative MRS would be extremely useful as part of the clinical routine practice as noninvasive identification of *IDH* mutation may influence surgical decision-making regarding the extent of the resection. Moreover, 2HG quantification may be useful for the identification of minor *IDH* mutations undetectable by immunohistochemistry, given that DNA sequencing is not available in all clinical centers. 2HG may also serve as a biomarker of *IDH* mutation if surgery is not feasible or associated with high risk, and as a marker of treatment efficacy, treatment response, and tumor progression, especially when evaluation based on other imaging methods is inconclusive, assisting clinicians in the choice of the best therapeutic strategy.

In vivo MRS methods

Since 2012, several in vivo ^1H MRS methods have been proposed for 2HG detection [9,10,11**]. These can be grouped into three types: short echo-time (TE) MRS [12]; long-TE MRS optimized for 2HG detection [13,14]; edited MRS [15,16*]. Examples of spectra acquired with these three methods have been shown previously [9].

Short-TE (TE = 30 ms) MRS is typically performed using the point resolved spectroscopy (PRESS) sequence, available on clinical scanners. This method has been shown to produce the highest rate of false-positives, which is the worst outcome for clinical evaluation [11**]. This is because the quantification of the 2HG signal at 2.25 ppm is complicated by the presence of other signals, including metabolites, macromolecules and baseline.

Long-TE MRS optimized for 2HG detection partially removes the overlap of 2HG at 2.25 ppm with other resonances, by employing the TE that provides minimal contribution of other signals. These methods require modified sequences and are rarely available in the clinic. Optimized PRESS uses $T_E = 97$ ms and has been shown to better quantify 2HG and reduce false-positive results compared to short-TE MRS [17]. Nevertheless, a recent study suggested that necrosis and treatment status were associated with false-positive 2HG measurements using optimized PRESS in GBM [18]. A semi-localization by adiabatic selective refocusing pulse sequence with $T_E = 110$ ms was also proposed as alternative approach for 2HG detection at 7 T [14].

Finally, edited MRS measures the 2HG signal at 4.02 ppm by completely removing the signals from other metabolites (Figure 2) and provided no false positives in a recent prospective 3 T study [16*]. These results point towards the need of implementation of advanced MRS methods for clinical examinations.

Both single-voxel (SV) MRS and MRS imaging (MRSI) have been employed for the detection of 2HG, yet the majority of the studies employed SV MRS, own to the shorter acquisition times and the higher reproducibility of the results with respect to MRSI.

Regardless the method employed, a few studies have reported lower sensitivity and specificity in post-operative compared to pre-operative cohorts [19,20]. This could be due to technical challenges related to the presence of post-surgical cavities, size of the residual tumor or effect of previous treatments. The evaluation of the full metabolic profile may thus become crucial for follow-up examinations and evaluation of tumor progression.

Correlation with cellularity and grade

Previous reports showed that 2HG concentrations detected by MRS were positively correlated with tumor cellularity [19,21]. 2HG concentrations were also found to be significantly different between low- and high-grade, consistent with differences in tumor cellularity in different grades [21] and confirming ex vivo findings obtained using ^1H high-resolution magic angle spinning (HR-MAS) spectroscopy [22]: mean ex vivo 2HG levels were shown to be significantly higher in grade IV compared to grade II gliomas. When normalized by average cellularity, 2HG levels did not differ significantly between grades II and IV, suggesting that 2HG differences between glioma grades reflect differences in

cellularity [22]. Conversely, another short-TE MRS 3 T study suggested that tumor grade had a significant influence on glutamate (Glu), but not on 2HG concentration [23]. This negative result could be due to the technical challenges associated with 2HG quantification using short-TE MRS, as discussed above.

In line with these findings, a recent study reported increased ex vivo 2HG levels in gliomas undergoing malignant progression from grade III to grade IV [24]. Additionally, 2HG levels were correlated with increased mitotic activity, axonal disruption, vascular neoplasia, and with several metabolites (see section “Correlation with other metabolites”) [24]. While in vivo studies did not report significant differences in 2HG levels between different histological subtypes [21,25], elevated ex vivo 2HG levels were reported in oligodendrogliomas undergoing progression to grade III and IV compared to astrocytomas [24].

Longitudinal changes

A 2HG concentration decrease during treatment has been shown previously [21,26,27**]. A prospective longitudinal study employing SV optimized PRESS at 3 T reported stable absolute 2HG levels in untreated patients with indolent disease, while 2HG showed a marked increase at progression. In contrast, 2HG levels decreased during radio- and chemotherapies in most patients under investigation, and the decrease during treatment was accompanied by a measurable decrease in tumor volume in almost all gliomas. 2HG concentration decreased more rapidly in oligodendrogliomas compared to astrocytomas or mixed gliomas, consistent with a better response to treatment of the first sub-type. In patients included up to 24 months after treatment, 2HG levels were very close to the detectability threshold set to 1 mM [21].

An edited 3D MRSI sequence was employed at 3 T to evaluate the effect of both standard treatments and a novel *IDH1* inhibitor on 2HG levels [26,27**]. 2HG levels measured relative to total creatine (creatine + phosphocreatine, tCr) were found to decrease significantly in patients receiving adjuvant radiation and chemotherapy and correlated with clinical status. Results from a phase 1 clinical trial indicated a rapid 2HG/tCr decrease by 70% after only 1 week of treatment with a novel *IDH1* inhibitor [27**].

Correlation with other metabolites

The median value of 2HG/tCr was found positively correlated with that of lactate(Lac)/tCr in pre-treatment scans, possibly resulting from the Warburg effect due to silencing of lactate dehydrogenase A (LDHA) in *IDH*-mutated gliomas, while it was negatively correlated with that of Glu + glutamine (Glx)/tCr in post-treatment scans, as a possible direct consequence of 2HG depletion during treatment [26]. These findings require further corroboration from larger patient cohorts. Similarly, the inhibition of *IDH1* activity through a novel anti-*IDH1* treatment was suggested to induce a reprogramming of tumor metabolism, showing decreased 2HG levels, reported relative to tCr quantified in healthy tissue (tCr_{healthy}), associated with increased Glx/ tCr_{healthy} , glutathione (GSH)/ tCr_{healthy} and Lac/ tCr_{healthy} levels. The trend to an inverse correlation between 2HG/ tCr_{healthy} and Glx/ tCr_{healthy} in *IDH*-mutated gliomas after treatment suggested the 2HG/Glx ratio as a more sensitive biomarker

of *IDH* mutation, given that Glu levels were found to be lower in *IDH1*-mutated compared to WT gliomas, as measured by the mass spectrometry in tumor biopsies [23]. However, the inverse correlation between 2HG and Glx in mutated gliomas has not yet been shown in vivo at baseline. Increased GSH levels after anti-*IDH1* treatment may lead to a reduced sensitivity of *IDH*-mutated gliomas to radiotherapy, providing evidence against a benefit of simultaneous administration of *IDH1* inhibitors and radiotherapy [27**]. The increase in Lac levels post-treatment were suggested to be linked with reversible downregulation of LDHA activity associated with inhibition of mutant *IDH1*.

A previous ¹H HR-MAS experiments also reported a positive correlation between 2HG and Lac levels in *IDH*-mutated recurrent glioma biopsies, as well as correlations of 2HG with several other metabolites commonly associated with tumor, such as free choline (Cho), phosphocholine (PCho), glycerophosphocholine (GPC), tCho, aspartate (Asp), gamma-aminobutyric acid (GABA), threonine (Thr), hypotaurine (hTau), creatine and phosphocreatine (Cr, PCr), betaine (Bet), glycine (Gly), GSH, phosphoethanolamine (PE), Glu, and Gln. Conversely, 2HG was negatively correlated with the *myo*-inositol (mIns)/tCho ratio, an index associated with the relative contribution of tumor cells and treatment-induced gliosis [22]. However, these correlations have not been yet corroborated by other ex vivo or in vivo studies. Correlations between 2HG and other metabolites observed both in vivo and ex vivo are summarized in Table 1.

Other metabolites

Differences in several other metabolites have been reported in *IDH*-mutated vs. WT gliomas, as well as across different glioma genetic sub-types and tumor grades, using in vivo and ex vivo MRS. Main findings are summarized in Table 2.

IDH vs. WT

IDH-mutated tumors exhibited a significantly lower Glu/tCr compared to WT gliomas from ultra-high-field short-TE MRS experiments [28]. The same study also reported a significantly lower GSH/tCr and significantly elevated tCho/tCr in mutated gliomas with respect to WT. However, a small cohort of patients was investigated and the basis set for metabolic quantification did not include the experimentally measured macromolecular spectrum, which may bias the results. Decreased Glu, Gln and GSH levels in *IDH*-mutated vs. WT gliomas were also reported from short-TE 3-T experiments [23]. However, the experimentally measured macromolecular spectrum was not used for the quantification, and Glu, Gln and GSH levels associated with a CRLB > 30% were excluded, possibly introducing a bias [29]. Significantly lower Glx levels and a trend to lower GSH levels were observed in a small cohort of *IDH*-mutated vs. WT GBM at 3 T [30]. Despite technical limitations, these results are in line with the reported lower Glu, Gln and GSH levels measured in mutant vs. WT *IDH* cell cultures [7]. Lower GSH levels in mutant *IDH* vs. WT may be explained by the selective depletion in these gliomas of nicotinamide adenine dinucleotide phosphate (NADPH), which is used by *IDH* for 2HG production and is thus less available for GSH recycling. However, lower total GSH levels in mutated gliomas were

not observed by liquid chromatography-mass spectrometry (LC-MS) in a recent study [31**].

Other more recent studies reported decreased Glu, but not Gln, levels in *IDH*-mutated glioma cells compared to WT, as well as in *IDH*-mutated vs. WT glioma tissue samples using gas-chromatography-mass spectrometry [23] and ¹H HR-MAS [24]. Lower GABA concentration was also observed in the *IDH*-mutated group in one of these studies [24].

Depletion of Glu levels were suggested to compensate for the altered flux of alpha-ketoglutarate to 2HG in *IDH*-mutated gliomas [23]. Significant differences in both Glu and Gln levels between *IDH*-mutated and WT glioma biopsies were observed in another study and were both linked to replenishment of alpha-ketoglutarate by glutaminolysis [32].

NAA and *N*-acetyl-aspartyl-glutamate (NAAG) levels were also found to be significantly lower in *IDH*-mutated vs. WT human glioma tissues, yet, the biological mechanism for NAA and NAAG depletion and whether it contributes to tumor pathogenesis remains unclear [7].

Very recently, maximum tCho/*N*-acetylaspartate (NAA) and tCho/tCr values measured using short-TE MRSI in lesions were shown to be significantly higher in *IDH*-mutated tumors compared to WT, whereas mean tCho/NAA and tCho/tCr were not significantly different between two groups. In addition, maximum tCho/NAA was significantly higher in grade IV gliomas compared to lower grade gliomas. In contrast, in the peritumoral area, the mean tCho/tCr was found to be significantly elevated in *IDH*-mutated vs. WT gliomas [33]. These results are in line with the elevated tCho/tCr measured in vivo in human mutated gliomas at 9.4 T [28], and with the observed higher levels of free Cho, GPC, and PCho in *IDH*-mutated vs. WT gliomas measured ex vivo using ¹H HR-MAS [24]. Given the very low concentration of free Cho in the brain, elevated tCho levels are very likely dominated by increased GPC and PCho. Lower PE and higher GPC levels in *IDH*-mutated vs. WT gliomas were reported in a study combining in vivo ³¹P MRSI in mouse xenograft models, ex vivo ³¹P HR-MAS measurements in human biopsies of *IDH*-mutated tumors, and cell line ³¹P MRS experiments, suggesting altered phospholipid metabolism caused by *IDH* mutation [34].

IDH1* vs. *IDH2

Higher 2HG levels in gliomas harbouring mitochondrial *IDH2* R172K mutations with respect to cytosolic *IDH1* R132H mutations were measured at 7 T [14], confirming previous cell culture findings [35]. The same group also reported higher 2HG/tCho and mIns/tCho, and a trend to increase in citrate/tCho and tCr/tCho, in *IDH2* vs. *IDH1*-mutated gliomas [36*]. However, given the variable tCho levels associated with *IDH* mutation, normalization of metabolic concentrations over tCho may represent a possible source of bias.

1p/19q codeletion

Higher cystathionine levels in brain tumors compared to normal tissue were previously reported from ex vivo tissue analysis [37,38]. A recent in vivo study reported significantly elevated cystathionine in patients with *IDH*-mutated 1p/19q codeleted gliomas compared

with their non-codeleted counterparts [31**]. In vivo results obtained using SV edited MRS (Figure 2) were corroborated by ex vivo LC-MS analysis in tumor samples. Ex vivo experiments showed significantly decreased levels of serine (Ser), Gly, Thr, and ornithine (Orn), and a trend for lower GSH in 1p/19q codeleted vs. non-codeleted tumors. Interestingly, cystathionine, Gly and serine are involved in the glutathione biosynthesis pathways and their altered levels were specifically attributed to the deletion of two serine- and cystathionine-pathway genes located on chromosome 1p - phosphoglycerate dehydrogenase (PHGDH) and cystathionine gamma-lyase (CTH) - possibly leading to a compensatory anti-oxidant mechanism in gliomas with 1p/19q codeletion (Figure 3) [31**]. Although cystathionine can be reliably detected using the same edited MRS protocol used for 2HG, possible further acquisition improvements have been reported [39*].

Grade and progression

Elevated in vivo tCho concentration was reported in high-grade compared to lower-grade gliomas [40,41], suggesting that tCho could be used as a marker of cellularity. However, since tCho is not a specific tumor cell marker, and its levels may change due to other pathological processes such as inflammation or gliosis, these results need to be taken with caution.

Choline containing compounds as measured using ¹H HR-MAS were found to be significantly higher in tumors that had undergone malignant progression [24]. Interestingly, oligodendrogliomas showed higher levels of GPC and tCho, while astrocytomas had higher levels of PCho and Cho. The associations of oligodendrogliomas with elevated GPC and astrocytomas with elevated PCho and Cho were suggested to reflect differences in the phospholipid metabolism found in the choline kinase pathway between these tumor subtypes. In the same study, positive correlations of 2HG levels with all choline-containing compounds were reported, corroborating previous findings suggesting that both 2HG and choline compounds levels are linked to cellularity. Given the reported differences of choline compounds between different tumor grades and histological subtypes (see below), care must be taken when reporting metabolic levels as ratios over tCho.

Several other metabolites measured using ¹H HR-MAS were found to be significantly elevated in gliomas undergoing malignant progression [24]. These included taurine (Tau), hTau, Gly, Glu, GSH, alanine (Ala), Asp, and Bet, Gln and glucose. 2HG was positively correlated with PE, GSH, Tau, Glu, Gln, Asp, myo-I, SI, GABA, PCr/Cr, Gly, Bet, and Thr. Among these metabolites, increases in PE, Glu and Gln were specifically associated with anaplastic astrocytomas, while Tau, Gly, Gln, and Bet were increased in anaplastic oligodendrogliomas.

Gln and Gly, which can be quantified using in vivo MRS, may be useful markers of tumor malignant progression. A very recent study reported a strong association between Gly levels measured by 3 T MRS and the presence of gadolinium enhancement. In addition, Gly levels, but not 2HG levels, were positively correlated with tumor cell proliferation as quantified by MIB-1 labelling index and high Gly/2HG was strongly associated with shorter survival [42**]. The correlation between Gly/2HG and patient outcome observed in this study may reflect the better outcome of *IDH* vs. WT gliomas (higher 2HG) combined with the better

outcome of 1p/19q codeleted vs. non-codeleted gliomas (lower Gly [31**]). However, no information on the codeletion status was reported.

Conclusions

Noninvasive 2HG detection has a strong clinical utility due to its high diagnostic and prognostic power. While conventional in vivo MRS methods available in clinical settings have shown low specificity to *IDH* mutation, the implementation of advanced in vivo MRS methods enabling reliable 2HG quantification is highly desirable for routine clinical examinations and follow-up of treatment response. Lower Glu, Gln and GSH levels seem to be associated with *IDH* mutation and abnormal production of 2HG, while elevated tCho suggests altered phospholipid metabolism, which may depend on tumor sub-type, grade and cellularity. Anti-cancer treatments have been suggested to affect tumor metabolism beyond 2HG production, yet the role of different metabolites in predicting patient outcome requires further elucidation. The recent discovery of cystathionine detectability in glioma using in vivo MRS opens up the possibility to exploit a potential novel biomarker of tumor response to treatment and progression. Although at the moment the quantification of the full metabolic profile does not have a direct implication for clinical evaluation, we believe that it provides a better understanding of the tumor pathophysiology and, in the future, it may be crucial for achieving higher specificity in glioma sub-type identification and for evaluation of tumor response to treatment and patient outcome.

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Key points

- Reliable 2HG quantification for clinical examinations requires implementation of advanced MRS methods.
- *IDH* mutation leads to tumor metabolic reprogramming beyond 2HG overproduction.
- Cystathionine and glycine may play a crucial role for glioma sub-type identification and follow-up of glioma progression.
- The evaluation of the full neurochemical profile may help achieving higher specificity in glioma sub-type identification and prediction of patient outcome.

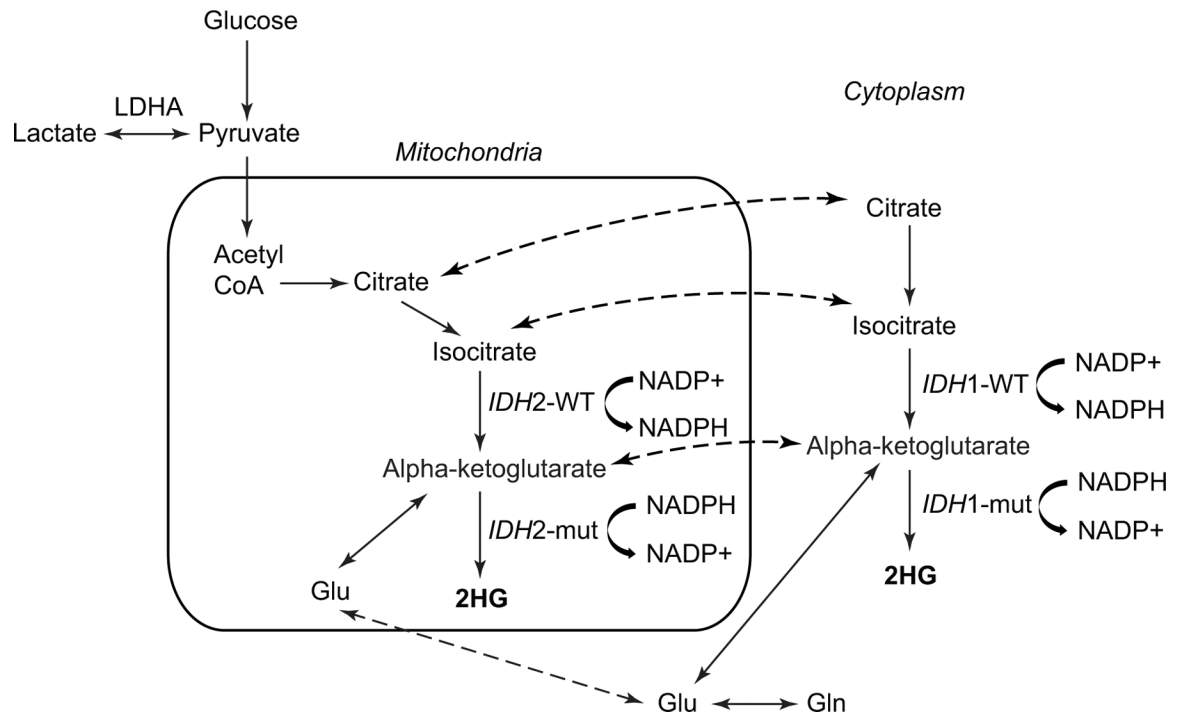


Figure 1: Schematic diagram of the 2HG metabolic pathway.

Illustration of *IDH* function change with cytosolic *IDH1* and mitochondrial *IDH2* mutations. *IDH1* and *IDH2* mutations do not occur at the same time. Dashed arrows indicate exchange of a metabolite between the mitochondria and the cytosol. Schematic based on previous publication [7].

2HG: 2-hydroxyglutarate; Glu: glutamate; Gln: glutamine; LDHA: lactate dehydrogenase A; NADP+: nicotinamide adenine dinucleotide phosphate; NADPH: nicotinamide adenine dinucleotide phosphate (reduced form); mut: mutated; WT: wild-type.

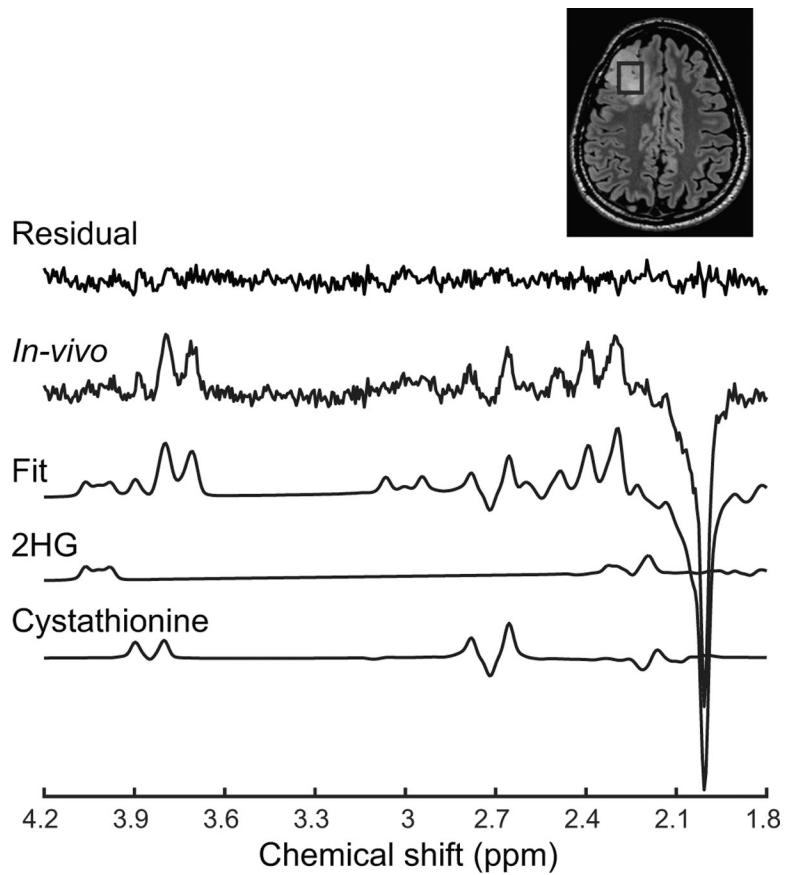


Figure 2: In vivo detection of 2HG and cystathionine.

An in vivo spectrum acquired in an *IDH1*-mutated, 1p/19q codeleted glioma is shown together with the LCMModel fit, the 2HG and cystathionine contributions, and residuals. The 2HG signal is visible at 4.02 ppm, while the cystathionine pattern is visible at 2.72 ppm. No line broadening was applied. The location and size of the VOI is shown on a FLAIR image. 2HG: D-2-hydroxyglutarate

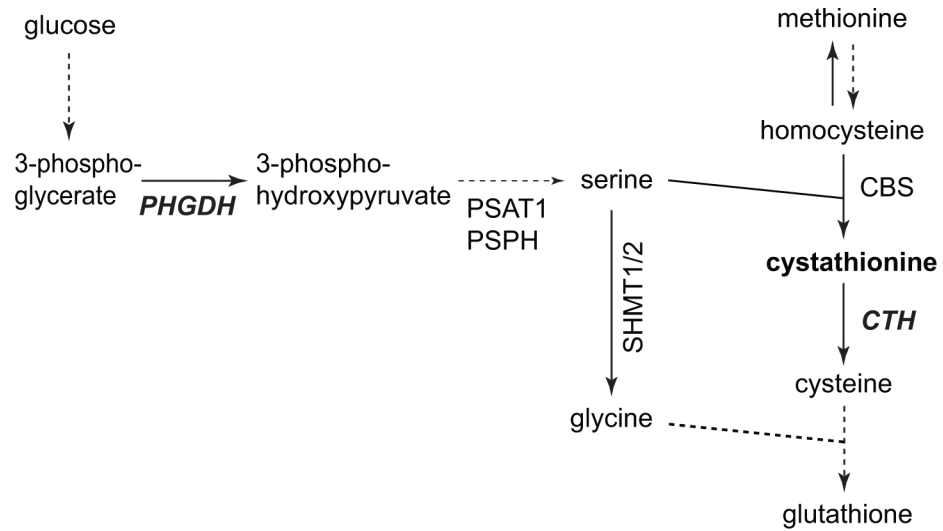


Figure 3: Schematic diagram of the cystathionine metabolic pathway.

Solid arrows = single metabolic step, dash arrows = multiple (not drawn) metabolic steps.

CBS: cystathionine-β-synthase; CTH: cystathionine gamma-lyase; PHGDH:

phosphoglycerate dehydrogenase; PSAT: phosphoserine amino transferase; PSPH:

phosphoserine phosphatase; SHMT1/2: Serine hydroxy methyl transferase 1/2.

Table 1:

Correlations of 2HG with other metabolites

	In vivo ¹ H MRS			Ex vivo ¹ H MAS
	Pre-treatment	Treatment		Recurrent tumors
		RT + CT	Anti-IDH1	
Positive correlations	Lac/tCr [26]			Asp, Bet, Cho, PCho, GPC, tCho, Cr, PCr, GABA, Gln, Glu, GSH, Gly, Lac, PE, Thr, hTau [22]
Negative correlations		Glx/tCr [26]	Glx/tCr _{healthy} , Lac/tCr _{healthy} , GSH/tCr _{healthy} [27]	mIns/tCho [22]

RT: radiotherapy; CT: chemotherapy; Lac: lactate; tCr: total creatine; tCr_{healthy} : tCr measured in the contralateral healthy tissue; Glx: glutamate + glutamine; GSH: glutathione; Asp: aspartate; Bet: betaine; Cho: free choline; PCho: phosphocholine; GPC: glicerophosphocholine; tCho: total choline; PCr: phosphocreatine; GABA: gamma-aminobutyric acid; Gln: glutamine; Glu: glutamate; Gly: glycine; PE: phosphoethanolamine; Thr: threonine; hTau: hypotaurine.

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Table 2:Currently known effects of *IDH* mutations and 1p/19q codeletion on the neurochemical profile

	In vivo ¹H MRS	Ex vivo tissue
<i>IDH</i> vs. WT	2HG ↑ [11] tCho/tCr ↑ [28, 33] Glu/tCr ↓, GSH/tCr ↓ [28] Glu ↓, Gln ↓, GSH ↓ [23] Glx ↓ [30]	2HG ↑ GPC ↑, PCho ↑, Cho ↑ [24] PE ↓ [34] Glu ↓ [23, 24, 32] Gln ↓ [32] GABA ↓ [24] NAA ↓, NAAG ↓ [7]
<i>DH2</i> vs. <i>IDH1</i>	2HG/tCho ↑ [14] mIns/tCho ↑ [36]	
1p/19q codeleted vs. non-codeleted	Cystathionine ↑ [31]	Cystathionine ↑ [31] Ser ↓, Gly ↓, Thr ↓, Orn ↓ [31]

2HG: 2-hydroxyglutarate; Cho: free choline; PCho: phosphocholine; GPC: glycerophosphocholine; tCho: total choline; tCr: total creatine; Gln: glutamine; Glu: glutamate; Glx: Glu + Gln; GSH: glutathione; GABA: gamma-aminobutyric acid; Gly: glycine; mIns: *myo*-inositol; NAA: *N*-acetylaspartate; NAAG: *N*-acetyl-aspartyl-glutamate; Orn: Ornithine; PE: phosphoethanolamine; Ser: serine; Thr: threonine.