Review

Advances and Challenges in Assessing 2-Hydroxyglutarate in Gliomas by Magnetic Resonance Spectroscopy: A Short Review

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ABSTRACT

The metabolite 2-hydroxyglutarate (2HG) accumulates in isocitrate dehydrogenase (IDH)-mutant gliomas and high-levels of 2HG can be non-invasively detected in living human brain by magnetic resonance spectroscopy (MRS). The concept of being able to detect a metabolite associated with an important gene mutation has generated considerable excitement in the fields of neurooncology and neuroradiology. However, challenges remain in reliably detecting 2HG before we can use it as a tool for making clinical decisions. In this review, we outline the advances and challenges in assessing 2HG by MRS.

Keywords:

2-Hydroxyglutarate, Gliomas, Magnetic Resonance Spectroscopy

Introduction

A groundbreaking study showed isocitrate dehydrogenase (IDH) mutations in about 10% of glioblastomas [1], and subsequent studies showed *IDH* mutations to occur in 50-80% of astrocytomas, oligodendrogliomas, and secondary glioblastomas [2-5]. The current understanding is that IDH mutations occur frequently, early in the cascade of astrocytomas and oligodendrogliomas [3], and is deeply involved in gliomagenesis. IDH mutations are known to be a powerful positive prognostic factor in World Health Organization (WHO) grade 3 and 4 [6,7]. IDH mutations in gliomas give rise to the metabolite 2-hydroxyglutarate (2HG) [8], which can be detected by magnetic resonance spectroscopy (MRS).

■ Functions of IDH and 2HG

The family of IDH enzymes includes three isoforms: IDH1, which is located in the cytoplasm, and IDH2 and IDH3, which localize in mitochondria **(Figure 1)**. IDH3, which uses the cofactor NAD^+ (as opposed to $NADP^+$ for IDH1 and IDH2) as the electron acceptor, converts isocitrate to α-ketoglutarate (α-KG) as part of the tricarboxylic acid (TCA) cycle.

IDH mutation is a gain-of-function mutation, in which 2HG is produced from α-KG **(Figure 1)** [9]. At least 5 point mutations of *IDH1* and 3 point mutations of *IDH2* have been reported in gliomas; *IDH3* mutation has not been reported in gliomas. The most commonly occurring point mutation in gliomas is *IDH1* R132H with a frequency of 85-95% [7,10], causing a missense mutation from Arginine (R) to histidine (H). 2HG is structurally similar to α-KG and acts as a competitive antagonist, causing inhibition of α-KG-dependent dioxygenases. These include the JmjC domain-containing histone demethylases

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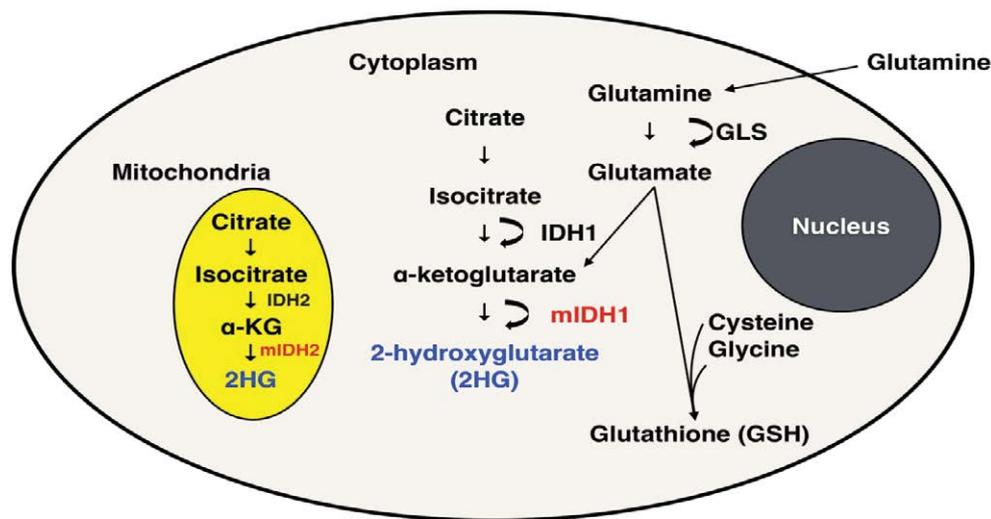
Figure 1: Metabolic changes in IDH-mutant gliomas.

Isocitrate dehydrogenase (IDH) physiologically converts isocitrate to α-ketoglutarate (α-KG). However, in IDH-mutant gliomas, α-KG is converted to the oncometabolite, 2-hydroxyglutarate (2HG). In IDH-mutant gliomas, extracellular glutamine is taken in and metabolized to form 2HG. Glutathione, an important metabolite which neutralizes oxidative stress, is made from glutamate.

(KDMs), which cause histone demethylation [11-13], and the ten-eleven translocation (TET) family of DNA hydroxylases, which cause DNA demethylation [13,14]. As a result of this competitive inhibition, diffuse DNA hypermethylation is noted in IDH-mutant gliomas [15], the so-called glioma-CPG island methylator phenotype (G-CIMP).

■ **Assessment of 2-hydroxyglutarate**

The 2HG molecule contains five nonexchangeable protons, giving rise to multiplets at three locations on MRS: approximately 4.02, 2.25, and 1.90 ppm [16]. The largest multiplet is located at 2.25 ppm. The detection of this multiplet is complicated by the spectral overlap of glutamate (Glu; 2.43 ppm), glutamine (Gln; 2.34 ppm), and gamma-aminobutyric acid (GABA; 2.28 ppm), which share the $^4CH_2$ group [17]. This can be expected given the structural similarities of Glu, Gln and 2HG **(Figure 2)**. Direct detection of the multiplet at 1.90 ppm is difficult due to its proximity to NAA resonance at 2.01 ppm, which shares $^3CH_2$. Also, a CH_3 peak of acetic acid at 1.90 ppm may further obscure the multiplet, especially in cases of pathologic state [18,19]. Finally, the multiplet at 4.02 ppm is partially overlapped with creatine (Cr: 3.92 ppm), phosphocreatine (PCr: 3.94 ppm), myoinositol (Ins; 4.06 ppm), lactate (Lac; 4.1 ppm) and free choline (fCh; 4.05 ppm), sharing $^2CH_2$ [16], which makes unambiguous detection of 2HG challenging **(Figure 3)**.

Furthermore, detection of 2HG is compounded by the fact that there are two enantiomers of 2HG, namely [D]-2HG and [L]-2HG, which cannot be distinguished by ^1H MRS [20]. IDH mutations produce only the [D]-2HG enantiomer, causing more than 100–fold increases [8,21]. In normal cells, both [D]-2HG and [L]-2HG are considered to be unwanted byproducts of cellular metabolism, and their intracellular levels are maintained at <0.1 mM [22]. However, recent studies have found that ischemia can cause up to 45-fold increases in [L]-2HG in cancer cells [23,24], so the presence of [L]-2HG must be taken into account when interpreting possible false-positive measurements of 2HG by MRS.

Also, there is a possibility that 2HG is produced in tumors with elevated glutamine metabolism. Teranuma *et al.* reported that 2 to 5 millimolar levels of 2HG, which is comparable to levels detected in IDH-mutant gliomas and acute myeloid leukemia (AML), was detected in IDH-wildtype breast cancer tissues with MYC pathway activation and increased glutamine metabolism [25]. This phenomenon has not been reported to be common in gliomas.

Assessing 2HG in serum, urine, and cerebrospinal fluid is another hot topic of clinical research. Detection of 2HG from body fluids would enable less invasive diagnosis of IDH-mutant gliomas and longitudinal assessment of treatment response in these tumors. Recent studies have
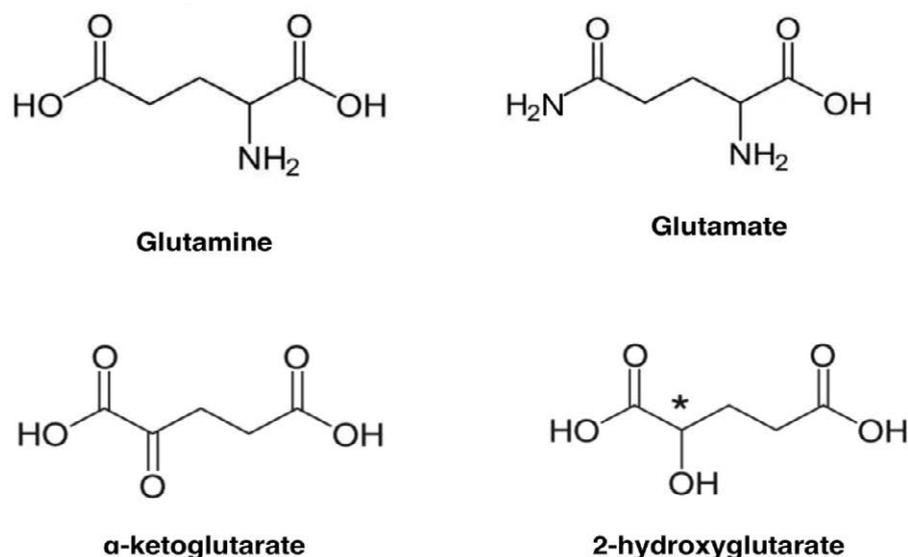
Figure 2: Structural similarities of glutamine, glutamate, α-ketoglutarate and 2-hydroxyglutarate.

Glutamine, glutamate, α-ketoglutarate and 2-hydroxyglutarate all share $^4CH_2$, leading to spectral overlap at a chemical shift of 2.25 ppm.
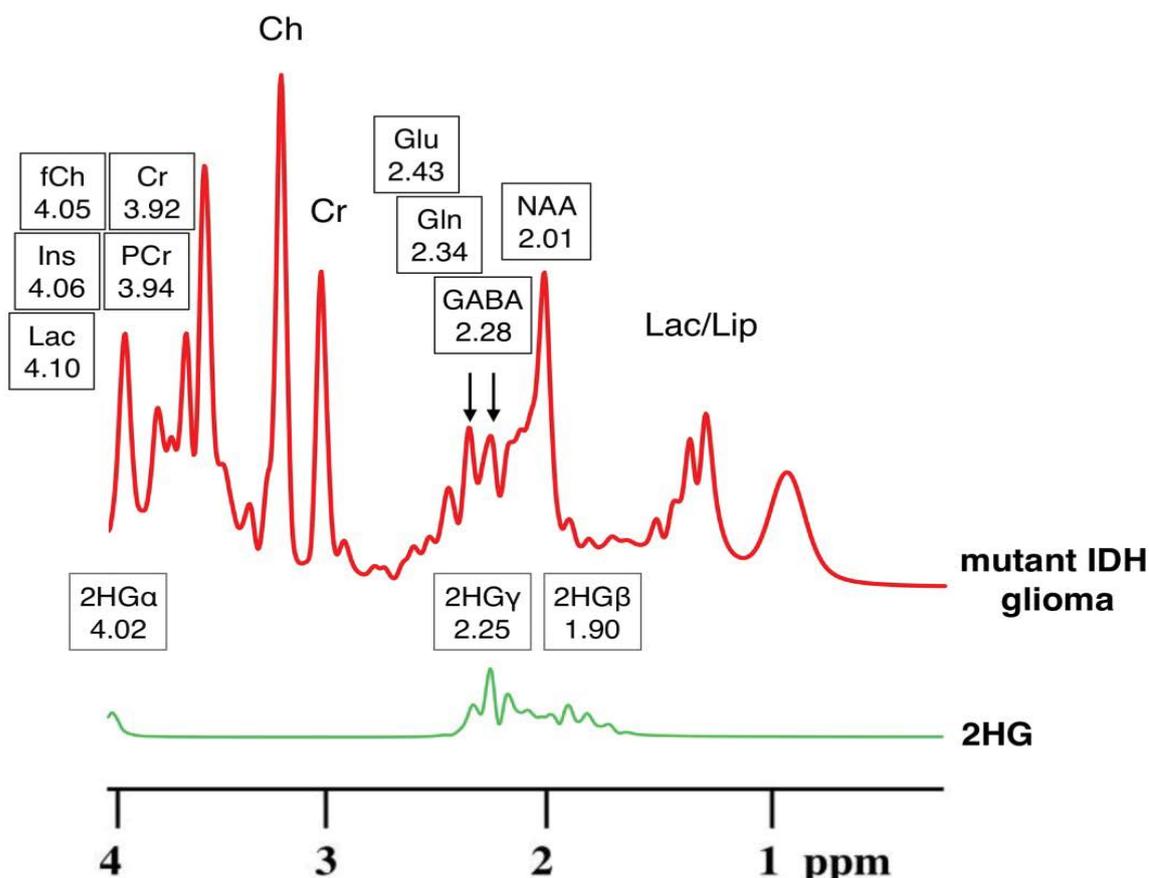

Figure 3: Spectral overlap of 2HG.

The detection of 2HGγ is complicated by the spectral overlap of glutamate (Glu; 2.43 ppm), glutamine (Gln; 2.34 ppm), and gamma-aminobutyric acid (GABA; 2.28 ppm). 2HGβ at 1.90 ppm is difficult due to its proximity to NAA resonance at 2.01 ppm. Finally, 2HGα at 4.02 ppm is partially overlapped with creatine (Cr: 3.92 ppm), phosphocreatine (PCr; 3.94 ppm), myoinositol (Ins; 4.06 ppm), lactate (Lac; 4.1 ppm) and free choline (fCh; 4.05 ppm), which makes unambiguous detection of 2HG challenging.

indicated that 2HG can be detected in cerebrospinal fluid [26,27] and urine [28], but it is still a challenge to assess 2HG in the serum of IDH-mutant gliomas [28,29]. It remains to see whether this can be overcome with more sensitive methods, or if 2HG accumulates in the serum of IDH-mutant glioma patients at all. The latter seems probable given that detection of 2HG in the serum of IDH-mutant AML patients has been well established [30-34]. An interesting study found that detection of circulating glioma cells is possible in 39% of glioblastoma patients using a cocktail of 5 antibodies, SOX2, tubulin beta-3, EGFR, A2B5, and c-MET. These circulating glioma cells were enriched for the mesenchymal signature [35]. IDH-mutant gliomas, which are known to be enriched with the proneural signature [36], may lack circulating tumor cells, thus contributing the absence of 2HG in the serum.

■ Methods of assessing 2-hydroxyglutarate by magnetic resonance spectroscopy

Long-echo MRS with TE at 97 ms with the use of three-dimensional volume-localized basis (VLB) spectra has been shown to be optimal for detection of 2HG [16,37]. A comparative study of PRESS sequences at short- (35 ms) and long- TE (97 ms) found long- TE to be superior by minimizing the effect of macromolecule signals [16]. Unambiguous detection of 2HG in mutant IDH gliomas was achieved by 2D correlation spectroscopy (COSY) [38-41] and J-difference spectroscopy [38]. In 2D COSY, the overlapping signals are resolved along a second orthogonal chemical shift dimension, and the cross-peaks resulting from the scalar coupling of Hα-Hβ protons show up in a region that is free of the contribution of other metabolites in both normal cells and wildtype-IDH tumors. While 2D COSY retains all of the metabolites in the spectrum, J-difference spectroscopy focuses on the metabolite of interest, such as 2HG. In the case of 2HG, the editing pulses tuned at around 1.9 ppm indirectly influence the line shape of the multiplet at around 4.0 ppm due to J-coupling by the spins. By subtracting the two spectra acquired at the same TE with and without editing pulses, removal of the contribution of overlapping metabolites Lac and Ins, which do not have their J-couple counterpart at about 1.9 ppm is possible, and the signal 2HGα signal can be detected at 4.02 ppm [38,42,43]. J-difference spectroscopy has historically been used in gliomas to subtract the lactate peak from lipid peak. 2D COSY has the highest resolving power to separate overlapping metabolites, but has less sensitivity and involves more complex quantification; J-difference spectroscopy has increased sensitivity, and quantification is straightforward, but is susceptible to subtraction errors. These methods are less available clinically and involve longer acquisition time [42]. Jafari-Khouzani and colleagues have reported MRS imaging (MRSI) of 2HG using a 3-tesla machine [44]. MRS using ultra-high magnetic field machines such as 7-tesla and higher, with a high signal-to-noise ratio, is able to better separate the 2HG peak at 2.25 ppm from those of Glu, Gln and GABA [45]. Preclinical trials involving intravenous injection of hyperpolarized ^{13}C-labeled α-KG to detect decreased glutamate production in IDH-mutant tumors [46], and hyperpolarized pyruvate to monitor the conversion of pyruvate to lactate, which is decreased in IDH-mutant tumors [47], presumably reflecting increase of metabolic substrate entering into TCA cycle. These techniques are outlined in three important review articles [42,43,48]. Previously reported methods to assess 2HG are also summarized in **Table 1**.

■ Data post-processing

We have achieved high sensitivity and comparable specificity in detecting 2HG in WHO 2/3 gliomas [49] and glioblastomas [50] with modulation of 2HG resonances by spectral fitting. Good spatial resolution, scanner stability, and absence of motion artifacts are required to reliably disentangle 2HG at 2.25 ppm. Currently available time- and frequency- domain analysis software includes Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES) in jMRUI [51] and CFIT [52] respectively. Another popular frequency domain approach is LCModel, which has been widely used to fit in vivo ^{1}H NMR spectra in humans and rodents. We used LCModel software (Stephen Provencher, Oakville, Ontario, Canada) [53] for spectral analysis. This software automatically adjusts the phase and chemical shift of the spectra, estimates the baseline, and performs eddy current corrections. Relative metabolite concentrations and their uncertainties were estimated by fitting the spectrum to a basis set of spectra acquired from individual metabolites in solution. To calculate the absolute metabolite concentrations, an unsuppressed water signal was used as a reference. The precision of spectral fitting is typically reported for individual metabolites in terms of Cramer-Rao lower bounds (CRLB) that

Table 1: Summary of in vivo MRS methods used to detect 2HG.

Method	Strengths and limitations	References
Short-echo MRS (PRESS, TE 30 ms)	Widely available for clinical use; high false positive rate	[21,49,50]
Long-echo MRS (PRESS, TE 97 ms)	Less false positives; relies on spectral editing to detect 2HG	[16,37]
7T MRS (PRESS TE 78 ms)	High signal-to-noise ratio; less available clinically	[45]
J-difference MRS	No false positives, increased sensitivity; less available clinically	[38]
2D-COSY	High specificity; less available clinically, complex quantification, low sensitivity, long acquisition time	[38-41]
Hyperpolarized ^{13}C, pyruvate	Assessment of metabolic flux; less available clinically	[46,47]
MRSI	Assessment of localization of 2HG; semi-quantitative	[44]

sets a lower bound on the variance of the fitting estimate. Quantification is considered unreliable when the CRLB, returned as a percentage of standard deviation (%SD), is over 20% [54]. To achieve precise spectral fitting, shimming should be performed so that full width at half maximum (FWHM) was less than or equal to 10 Hz.

■ Other metabolic changes detected in IDH mutant gliomas

Significant reduction of Glx (the sum of glutamate (Glu) and glutamine (Gln)) in WHO grade 2-4 IDH mutant gliomas [49,50,55] and reduced glutathione (GSH) in WHO grade 2-3 IDH mutant gliomas [49] compared to IDH wildtype gliomas. *In vitro* studies which placed isotope labeled glutamine into media growing IDH-mutant cells showed that the labeled carbon is ultimately used by 2HG [8], suggesting that 2HG is primarily derived from glutamine in IDH-mutant gliomas **(Figure 1)**. Glutamine is hydrolyzed by glutaminase to produce glutamate, which is subsequently converted to α-KG [8,56]. Glutaminase inhibitors such as bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide) (BPTES) [56] and 6-diazo-5-oxo-L-noleucine (DON) [57] have been shown to be effective in *IDH1-R132H* expressing cells. Furthermore, a study injecting hyperpolarized ^{13}C α-KG into rats injected with IDH-mutant glioblastoma cells found that glutamine production is reduced in mutant-IDH gliomas, mainly due to the decrease of branched-chain amino acid transaminase 1 (BCAT1) enzyme, which catalyzes the transamination of branched-chain amino acids while converting α-KG to glutamate [46]. A report from Nagashima *et al.*, suggested that elevated 2HG as well as decreased Glu is diagnostic for IDH-mutant gliomas [55]. GSH, an important antioxidant, is a tripeptide formed from Glu, as well as

cysteine and glycine **(Figure 1)**. This metabolite was also decreased in WHO grade 2/3 gliomas [49]. This is in agreement with preclinical studies showing that *IDH1*-mutation in gliomas depletes GSH production [58,59], rendering IDH-mutant gliomas sensitive to radiation [60,61]. Susceptibility to reactive oxygen species may be another reason for the relatively good survival in IDH mutant gliomas. Furthermore, a metabolomic study looking at IDH1 and IDH2 mutations showed reduction of N-acetyl-aspartyl-glutamate (NAAG) in cells expressing both IDH1 and IDH2 mutation [62].

■ Clinical relevance and future implications

Quantitative measurement of 2HG by MRS has potential therapeutic implications, although an almost 100% sensitivity and specificity in detecting IDH mutations would be necessary to make therapeutic decisions based on this measurement. Reliable detection of 2HG by MRS would make non-invasive, preoperative analysis of IDH mutations in glioma patients possible. Second, determination of therapeutic response after surgery and chemoradiotherapy is possible by serial evaluation of 2HG [63,64]. Mutant IDH1 inhibitors, which are known to deplete 2HG, are being evaluated in clinical trials for mutant-IDH gliomas [65-69], so monitoring of therapeutic response by MRS would be rational. Third, since all IDH mutations are known to produce 2HG [70], detection of rare *IDH1* and *IDH2* mutations are possible. Fourth, for select cases of gliomas near eloquent areas, total removal of the tumor may be feasible after neoadjuvant chemotherapy [71] or radiation. Since no other brain tumor besides gliomas, including ependymomas and medulloblastomas, have IDH mutations [4], detection of 2HG is diagnostic for IDH-mutant gliomas, thus a

biopsy for pathological diagnosis may be omitted. A clinical report indicates the sensitivity of secondary glioblastomas to temozolomide [72], and is a candidate for neoadjuvant chemotherapy in mutant-IDH gliomas. Also, preclinical studies IDH1 R132H mutations render cells to be more sensitive to radiation [60,61]. Furthermore, a retrospective analysis suggests a survival benefit for radical surgery, including FLAIR hyperintensity areas, in mutant-IDH but not wildtype-IDH gliomas [73]. Preoperative evaluation of 2HG by MRS may be helpful in determining indication for radical surgery.

IDH mutation triggers widespread effects, including histone and DNA hypermethylation and metabolic changes such as increased glutamine metabolism and decreased glutathione production. Recent evidence points to the production of 2HG as the main cause of these effects. Furthermore, new treatments targeting IDH mutations are currently being evaluated. Technical advances are being made to reliably detect 2HG by MRS in glioma patients which will lead to making clinical decisions with this data.

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