Published in final edited form as:

Top Magn Reson Imaging. 2017 February ; 26(1): 27–32. doi:10.1097/RMR.0000000000000113.

Interrogating IDH Mutation in Brain Tumor: Magnetic Resonance and Hyperpolarization

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Abstract

Magnetic resonance spectroscopy (MRS) offers the possibility to non-invasively quantify 2HG concentration in the brain in the clinic, thereby serving as a valuable tool for patient-stratification as well as targeted treatment monitoring. Recently, hyperpolarized magnetic resonance techniques have opened up new opportunities for metabolic imaging not possible with conventional magnetic resonance spectroscopy in the brain. With over 10,000-fold increase in signal-to-noise ratio (SNR), dynamic metabolic processes can be interrogated *in vivo* with very high specificity by hyperpolarized MRI. In the following article, we will review relevant clinical studies and practical considerations of MRS and hyperpolarized MRS, as well as discuss some promising preclinical hyperpolarization studies to interrogate real-time metabolism in IDH mutations *in vivo*.

Keywords

IDH mutations; 2HG; α -KG; ¹H MRS; hyperpolarization; ¹³C MRS; glioma

1. Isocitrate Dehydrogenase1/2 (IDH1/2) mutations lead to production of 2-hydroxyglutarate (2HG)

Single allele point mutations of isocitrate dehydrogenase 1 (IDH1) and isocitrate dehydrogenase 2 (IDH2) genes occur frequently in low/intermediate-grade gliomas (around 80%, WHO grade II and III) and secondary glioblastomas (85%, WHO grade IV), constituting a distinct subtype of brain cancer with better patient prognosis [1, 2]. Around

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85% of IDH1 mutations occur at codon 132 with the substitution of arginine to histidine (R132H). Wild-type (wt) IDH1/2 enzymes catalyze oxidative decarboxylation of isocitrate to α -ketoglutarate (α -KG). Mutated IDH enzymes, however, perform NADPH-dependent reduction of α -KG to R(-)-2-hydroxyglutarate (2HG), an oncometabolite whose concentration can increase over 100-fold in cancer tissues [3, 4].

Magnetic resonance spectroscopy (MRS) offers the possibility to non-invasively quantify 2HG concentration in the brain, thereby serving as a valuable tool for patient-stratification as well as targeted treatment monitoring [5]. Recently, hyperpolarized magnetic resonance techniques have opened up new opportunities for metabolic imaging not possible with conventional magnetic resonance spectroscopy in the brain [5]. With over 10,000-fold increase in signal-to-noise ratio (SNR), dynamic metabolic processes can be interrogated *in vivo* with very high specificity by hyperpolarized MRI. In the following sections, we will review relevant clinical studies and practical considerations of MRS and hyperpolarized MRS, as well as discuss some promising preclinical hyperpolarization studies to interrogate real-time metabolism in IDH mutations *in vivo*.

2. *In vivo* 2HG detection with Magnetic Resonance Spectroscopy (MRS)

2HG molecules is a strongly coupled spin system with five non-exchangeable proton resonances at 4.02 ppm (H2), 2.25 ppm (H4, H4') and 1.9 ppm (H3, H3') at pH 7 (Figure 1a, Table 1). *In vivo* detection of 2HG is challenging due to spectral overlap with NAA (2.01 ppm) and glutamate/glutamine (Glu/Gln) / *gamma*-Aminobutyric acid (GABA) moieties (2.1–2.4 ppm) [6–9].

Current strategies for 2HG detection largely fall into three categories: **I**). Detection of H4 with single voxel point resolved spectroscopy (PRESS) or adiabatic selective refocusing (LASER, semi-LASER), **II**) Detection of H2 with spectrum editing approach such as Meshcher-Garwood PRESS (MEGA-PRESS) or MEGA-LASER, and **III**) Detection of H2/H3 cross peak with 2D correlation spectroscopy (COSY) [7–11]. Key experimental parameters and results in recent clinical studies using these methods are summarized in Table 2.

The single voxel double spin echo (PRESS) sequence consists of a slice-selective 90 degree excitation pulse followed by two slice-selective orthogonal 180 degree refocusing pulses. In conventional single voxel MRS at short echo time of 30ms, the spectral overlap of much higher concentrations of glutamate (Glu, 12mM), glutamine (Gln, 5mM) and N-acetyl (NA, 12mM) makes it difficult to sort out the much lower concentration of 2HG resonance at 2.25ppm (ca. 3–5mM), even with prior knowledge fitting [12]. An optimized long echo time PRESS reported by Choi *et al* at TE=97ms (TE1/TE2 = 32/65 ms) minimized the contribution of Glu and Gln [9]. Choi *et al* was able to demonstrated 100% detectability of 2HG in IDH mutations (n=30, 15 with IDH1/2 mutation) [8]. Similarly, la Fuente *et al* conducted a study with more patients (n=89, 80 with IDH mutations) and slightly different TE1/TE2 timing (26/71 ms) highlighted the importance of voxel size selection. When the voxel size is less than 3.4 mL, sensitivity is only around 8% (2/24) but surges dramatically to

91% (20/22) after increasing the voxel size to at least 8 mL for patients with larger brain tumors [10].

By taking advantage of J-couplings, spectrum difference editing sequences can be optimized to detect specific metabolites while removing signals from unwanted, overlapping metabolites. MEGA-PRESS, for example, builds upon the PRESS sequence by augmenting the second refocusing pulse with a new pair of 180 pulses, one on each side. Based on the strongly coupled H3 and H2 protons of 2HG, by suppressing the H3 resonance using the, narrow band frequency selective 180 degree RF pulses (at 1.9 ppm, ON) in one scan and on the second scan at 7.5ppm (OFF). The difference between the two scans results in H2 resonance at 4.02 ppm without significant interference from other metabolites (Figure 1b). The echo time in this case should be optimized around $1/2J$ or $1/J$ (coupling constant). Choi *et al* optimized MEGA-PRESS to have TE of 106 ms (20/86 ms) while Andronesi *et al* used a different localization pulse sequence, LASER, with TE of 75 ms [8, 9]. One disadvantage of difference editing, however, is the lower signal yield (less than 50%) compared with that of PRESS sequence alone [9]. Other spectrum editing sequences such as JPRESS (TE-averaging PRESS), which is commonly used in MRS of GABA, could also be applied in the context of 2HG detection without much decrement in signal-to-noise ratio (SNR).

To further separate out the 2HG spectra, two dimensional MR methods can be employed. LASER-COSY introduces a second dimension so that metabolites can be identified via less crowded cross-peaks. For 2HG, the cross peak of H2 and H3 at around 4.02/1.9 ppm lies in a region where no other metabolites from normal tissues or wild-type (wt) IDH cancer tissues resonate, thereby uniquely identifying 2HG (Figure 1c and 1d). Although 2D methods have been deployed in clinical studies, they typically require longer scan times and a larger voxel size of 27 mL compared to that of 1D methods [8, 10].

Post-acquisition processing typically consists of properly combining data from multiple receiving channels, zero-filling, apodization, and using spectral-fitting tools to extract the concentration of 2HG and other metabolites of interest with unsuppressed water references [6, 8–10]. Table 2 lists the typical assumptions used for spectrum quantification. Alternatively, ratios of 2HG to other metabolites have been reported. The ratio of 2HG/ (Glu +Gln) tends to be more effective compared with 2HG/Cr, because creatine levels vary in cancerous tissues.

3. Preclinical hyperpolarization trials to interrogate IDH pathway

In the study conducted by Chaumeil *et al* [13], U87 glioblastoma (GBM) cells were transduced with a lentiviral vector expressing either wild-type (wt) or mutant (mut) IDH1 and cell lysates were prepared. The lysates were injected with hyperpolarized ^{13}C -labeled α -KG and scanned with ^{13}C MRS on an 11.7T MR spectrometer for 300 seconds. The resulting spectra showed a peak at 183.9 ppm in the IDH1-mut cell lysates, but not in the IDH1-wt cell lysates. This resonance was confirmed to be ^{13}C -labeled 2-HG in complimentary 2D ^1H - ^{13}C MRS experiments. To demonstrate that hyperpolarized α -KG could penetrate the blood-brain barrier and be delivered to tumors before the signal decayed, U87-IDH mut cells and U87-IDH wt cells were injected orthotopically into the brains of

nude rats. When the tumors reached approximately 0.4 cm³ the rats were injected intravenously with hyperpolarized ¹³C-labeled α-KG and scanned with ¹³C MRS on a 3T clinical MRI. Because of the low field strength, the α-KG and 2-HG resonances, which differ by only 0.1 ppm, could not be spectrally resolved. However, the spectra from the rats harboring IDH1-wt xenografts displayed a unimodal temporal evolution, representing only the resonance from α-KG, whereas the spectra from the rats harboring IDH1-mut xenografts displayed a bimodal temporal evolution, representing the convolution of resonances from α-KG and 2-HG. The spatial distribution of the metabolites was determined with ¹³C 2D chemical shift imaging (CSI) (Figure 2). Voxels were placed in areas with blood vessels, normal brain, and in the IDH1-wt and IDH1-mut tumors. 2-HG was only observed in the IDH1-mut tumor voxels, with an average SNR of 1.4, demonstrating the validity of this technique. The SNR could potentially be increased by employing ester derivatives of α-KG as hyperpolarization substrates which are shown to have a greater uptake in the cell [14].

In another study performed by the same laboratory [15], hyperpolarized ¹³C-labeled α-KG was used to detect decreases in glutamate production in IDH1-mut tumors. Branched-chain amino acid transaminase 1 (BCAT1) is responsible for converting branched-chain L-amino acids into branched-chain α-keto acids while simultaneously converting α-KG into glutamate, and because the expression of the BCAT1 enzyme is significantly reduced in IDH1-mut tumors compared with IDH1-wt tumors [16], corresponding glutamate levels are expected to be reduced. Using spectrophotometry, Western blotting, and quantitative real-time polymerase chain reaction (qRT-PCR) assays of IDH-wt and IDH-mut cells and xenografts, the investigators showed that BCAT1 activity, protein levels, and mRNA expression were significantly decreased in IDH1-mut cells compared with IDH-wt cells. In addition to BCAT1, the aspartate transaminase (AST) and glutamate dehydrogenase (GDH) enzymes, which help catalyze the conversion of α-KG to glutamate, were also reduced in IDH1-mut cells. In the cell lysate experiments, hyperpolarized α-KG was analyzed by NMR spectroscopy and glutamate accumulation was observed at 177.5 ppm within 12 seconds in the IDH1-wt cells. However, in the IDH1-mut cells, the glutamate resonance was barely visible. This result was also seen *in vivo* with intracranial rat xenografts harboring the IDH1-wt and IDH1-mut cells. Using a clinical 3T MR scanner, hyperpolarized ¹³C-labeled α-KG was injected intravenously and the relative glutamate concentration was measured using ¹³C 2D CSI pulse sequences with voxels placed in normal brain tissue and in tumors. 25 seconds after injection, glutamate was present in normal brain tissue and in the IDH1-wt tumors, but not in the IDH1-mut tumors. Taken together, the findings from these studies indicate that injection of ¹³C-labeled α-KG results in increased signal of hyperpolarized 2-HG yielding positive contrast enhancement, and therefore direct confirmation of the presence of IDH1 mutations. On the other hand, injection of ¹³C-labeled α-KG results in decreased signal of hyperpolarized glutamate, yielding negative enhancement, and therefore only indirect confirmation of the presence of IDH1 mutations. There are current efforts in place to simultaneously detect both the increase in 2-HG and decrease in glutamate from one injection of hyperpolarized α-KG.

Hyperpolarized pyruvate has also been used as an imaging agent in profiling the metabolism of IDH-mutated gliomas [17]. Several studies have monitored the conversion of hyperpolarized pyruvate to lactate in glioblastoma tumors *in vivo* for the purposes of

diagnosis [18, 19] and treatment response [20–22]. Two cell lines were studied: U87 which is a well-established glioblastoma cell line and BT142 which is a glioma cancer stem cell line derived from a patient with a WHO grade III IDH1-mutant oligoastrocytoma. Western blot analysis showed that the expression of lactate dehydrogenase A (LDHA) and monocarboxylate transporter 1 and 4 (MCT1 and MCT4) was significantly reduced in the BT142 cells compared with U87. Hyperpolarized ^{13}C -labeled pyruvate was injected into the medium, and the cells were collected and analyzed with ^{13}C MRS on an 11.7T spectrometer for 300 seconds. The metric used to measure the flux from pyruvate to lactate was the ratio of the area under the time-dependent signal curve of lactate to that of pyruvate, denoted as $[\text{Lac}]/[\text{Pyr}]$. The value of $[\text{Lac}]/[\text{Pyr}]$ was significantly lower in BT142 cells compared with the U87 cells. In corresponding *in vivo* experiments, U87 or BT142 were orthotopically injected into the brains of athymic mice. When tumors were approximately 100 mm³ in size, mice were injected intravenously with hyperpolarized ^{13}C -labeled pyruvate and scanned on a 14.1T animal MRI. U87 and BT142 had similar levels of pyruvate uptake, but the BT142 tumors produced significantly less hyperpolarized lactate than did the U87 tumors. The mice harboring BT142 xenografts were then treated with Temozolomide (TMZ), which is a standard-of-care chemotherapeutic agent for glioblastoma. The size of the tumors was dramatically reduced and survival was increased significantly. However, there was no appreciable difference in $[\text{Lac}]/[\text{Pyr}]$ over the course of treatment. Thus, due to the reduced levels of LDHA, MCT1 and MCT4, hyperpolarized pyruvate did not appear to be a good option for imaging IDH1-mutated tumors. Of course only one cell line was tested and results with other lines may yield different results. This is an active area of current research and more research is needed to link genomics and metabolomics of IDH mutation with real-time metabolic imaging in brain tumor with hyperpolarization.

Conclusion

In summary, multiple proton MRS techniques are in place and has been applied in the clinic to for detection of 2HG and follow up of therapy in brain tumor patients with IDH mutation. On the other hand, real-time follow up of IDH metabolic pathway with hyperpolarized MR is relatively recent and has been only applied to animal models. However, hyperpolarized ^{13}C MR has been rapidly gaining acceptance in the clinic and various clinical trials in multiple organ sites, including brain, are in progress in at least eight institutions worldwide to establish the clinical efficacy of this emerging technique. It is plausible that *in vivo* metabolic imaging in IDH mutation is the next frontier in brain cancer diagnosis and assessing therapeutic efficacy; with the combined knowledge of genomics a clearer understanding of tumorigenesis in brain might be achieved.

Acknowledgments

Sources of Support:

We also thank Brain Tumor NCI SPORE 2P50CA127001, NCI R21CA185536, Koch Foundation, CPRIT-RP150701, MD Anderson IRG grants and MD Anderson startup for funding support.

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Figure 1.

a) Simulated PRESS 1H spectrum of 2 hydroxyglutarate (2HG) at 3T MR scanner using VeSPA simulation platform. TE=97 ms (32/65 ms) and line width = 1 Hz. In practical situations, linewidth is usually on the order of 8–12 Hz. **b)** Simulated MEGA-PRESS spectrum of 2HG at 3T with the edited peaks at around 4.02 ppm, line width = 8 Hz. **c)** 2D LASER-COSY spectrum from a patient with IDH mutation showing the cross-peak 4.02/1.9 ppm that uniquely identifies 2HG. Voxel size = $3 \times 3 \times 3$ cm [8]. **d)** A vertical slice of the 2D spectrum at $\delta_2 = 4.02$ ppm showing 2HG peaks [7].

Figure 2. MRI images of mice bearing U87IDH1-mut (top row) and U87IDH1-wt (bottom row) tumors. **a)** T2 images with the tumors outlined. **b)** 2D CSI image depicting the uptake of hyperpolarized α -KG. There are similar levels of uptake in both the U87IDH1-mut and U87IDH1-wt tumors. **c)** 2D CSI image acquired at the same time as (b) showing production of hyperpolarized 2-HG in the U87IDH1-mut tumor, but not in the U87IDH1-wt tumor.

Table 1

J coupling constants among the five non-exchangeable protons for R(-)-2-hydroxyglutarate (2HG) that can be used with spectrum simulation with VeSPA simulation platform.

Proton Position	J coupling in (Hz)
2, 3	7.6
2, 3'	4.1
3, 3'	-14.0
3, 4	5.3
3, 4'	10.4
3', 4	10.6
3', 4'	6.0
4, 4'	-15.0
	pH = 7.0

Table 2
Summary of clinical studies related to *in vivo* R(-)-2-hydroxyglutarate (2HG) detection

Sequence	TE (ms)	Average	Voxel (cm ³)	Scan time (mins)	Line width (Hz)	Patient Info	Results	Detection Limit	Quantification	Source
PRESS	30	128	2 × 2 × 2	4.3	< 30	n = 24 (9 with IDH1 mut)	All of the 9 mutant tumors had measurable 2HG level and low CRLB [†] ; 4 potential false positives with high CRLB.	NA	Assuming water conc. of 35 M	[5]
PRESS	97 (TE1/2 = 32/65)	64	2 × 2 × 2	2.1	< 6	n = 30 (15 with IDH1/2 mut)	All of the 15 patients with 2HG all had IDH1/2 mutation. No detectable 2HG for wild type IDH patients.	1.5 mM	Assuming water conc. of 42.3 M	[7]
PRESS	97 (TE1/2 = 26/71)	128 or 256	see results	5.5 or 9.5	4–8	n = 89 (80 with IDH1/2 mut)	Sensitivity of 91% if voxel > 8ml; Sensitivity of 8% if voxel < 3.4ml	1.0 mM	Assuming water conc. of 43.3 M	[8]
MEGA-PRESS	106 (TE1/2 = 26/80)	384	2 × 2 × 2	13	< 6	n = 30 (15 with IDH1/2 mut)	All of the 15 patients with measurable 2HG all had IDH1/2 mutation.	1.5 mM	Assuming water conc. of 42.3 M	[7]
MEGA-LASER	75	200	3 × 3 × 3	5	6–12	n = 10 (2 with IDH1 mut)	Both IDH1 mutant patients had measurable 2HG level.	2.0 mM	Ratio of 2HG/(Glu+Gln)	[6]
2D LASER-COSY	45 (64 t ₁ increment of 0.8 ms)	8 per t ₁ increment	3 × 3 × 3 or 3.5 × 3.5 × 3.5	12.8	6–12	n = 10 (2 with IDH1 mut)	Both IDH1 mutant patients had measurable 2HG level.	2.0 mM	Ratio of 2HG/(Glu+Gln)	[6]
2D COSY at 7T	20 (64 t ₁ increment of 0.4 ms)	8 per t ₁ increment	8.8 – 18	17	NA	n = 9 (2 with IDH1 mut)	2HG detected in both patients with IDH1 mutation.	NA	Ratio of 2HG to Cr	[9]

[†]CRLB = Cremer Rao lower bound