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2-Hydroxyglutarate as a Magnetic Resonance Biomarker for Glioma Subtyping[1]

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Abstract

Mutations in the *isocitrate dehydrogenase* (*IDH*) genes are frequently found in gliomas and in a fraction of acute myeloid leukemia patients. This results in the production of an oncometabolite, 2-hydroxyglutarate (2-HG). Glioma patients harboring IDH mutations have a longer survival than their wild-type counterparts. 2-HG has been detected noninvasively in gliomas with IDH mutations using magnetic resonance spectroscopy (MRS), suggesting its potential clinical relevance for identifying glioma subtypes with better prognosis. In this paper, the recent developments in the MRS detection of the 2-HG in gliomas are reviewed, including the therapeutic potentials and translational values.

Translational Oncology (2013) 6, 92–98

Introduction

Somatic mutations in the genes *isocitrate dehydrogenase 1* (*IDH1*) and *IDH2* have been identified in a subset of gliomas, acute myeloid leukemia, and less frequently in other malignancies [1–4]. These mutations result in the substitution of the arginine 132 (in IDH1) and arginine 172 (in IDH2) codons by histidine, causing alterations in the normal enzymatic activities of IDH1-R132 and IDH2-R172 [1]. IDH mutations are associated with alterations in DNA methylation and impede the oxidative decarboxylation of isocitrate, resulting in overproduction and accumulation of the oncometabolite 2-hydroxyglutarate (2-HG) instead of α-ketoglutarate [1,5,6].

Being a direct and noninvasively detectable metabolic consequence of a genetic mutation in cancer, 2-HG detection in glioma serves as a unique biomarker for identifying *IDH* mutations. The potential diagnostic value of 2-HG as a biomarker of patient survival is further confirmed by better survival in patients with IDH mutations than wild type. *IDH* mutations have been reported in more than 70% of low-grade gliomas [World Health Organization (WHO) grades II and III] and secondary glioblastomas (Table 1), while the frequency of IDH mutations in primary glioblastoma is much lower (<10%) [3,7,8]. Secondary glioblastomas may often be incorrectly diagnosed as primary glioblastomas [9]. Hence, the detection of 2-HG could be a potential tool for *in vivo* distinction of secondary from primary glioblastomas [10]. The molecular pathogenesis of *IDH1/2* mutations in the development of gliomas has yet to be identified. However, such identification may improve our understanding of the mechanisms of glioma development and may lead to the development of novel molecular classification and therapy. 2-HG being a magnetic resonance (MR)–visible indicator of IDH mutation offers unique possibilities in monitoring and tracking glioma patients.

Both IDH1 and IDH2 are NADP+-dependent (oxidized form of nicotinamide adenine dinucleotide phosphate) enzymes catalyzing the conversion of isocitrate to α-ketoglutarate (Figure 1). In mutant tumors, the NADPH levels are decreased, causing a potential effect in cellular biosynthetic processes, such as lipogenesis [2]. By metabolic profiling, more than 200 metabolites have been analyzed in human oligodendroglioma cells engineered to express mutant *IDH1/IDH2* [11]. The major biologic alterations identified were increased levels of free amino acids and lipid precursors and depletion of metabolites involved in the tricarboxylic acid cycle (also known as Krebs cycle): citrate, α-ketoglutarate, fumarate, *cis*-aconitate, and malate [11].

MR spectroscopy (MRS) has been widely used as a quantitative analytical tool for monitoring metabolism in different types of malignancies [12,13]. Metabolic profiling helps in assessing pathophysiological processes associated with tumor biology and is a valuable monitoring tool for developing novel therapeutic anticancer agents

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Table 1. Frequency of IDH Mutations in Various Glial Brain Tumors.

WHO Grade	Glioma Type	*n*	IDH Mutation Status			References
			IDH1	IDH2	Combined (%)	
II	A	405	282	4	74	[3,4,6,7,24,35]
	OA	196	150	1		
	O	363	268	8		
III	A	398	257	4	76	[3,4,6,7,24,35]
	OA	279	229	11		
	O	245	184	12		
IV	GBM secondary	85	69	0	82	[3,6,7,9]
IV	GBM primary	673	32	0	5	[3,6,7,9]

Abbreviations: A, astrocytoma; OA, oligoastrocytoma; O, oligodendroglioma.

[12]. More recently, promising results have been obtained using this technique for 2-HG detection *in vivo* [14,15]. Crucial to these approaches were the determination of whether the accumulation of 2-HG in *IDH*-mutated gliomas was within the range of MRS-detectable levels and in the precise assignment of 2-HG resonances overlapping with other metabolites using conventional one-dimensional (1D) MRS. This mini-review highlights the recent developments in the detection of the 2-HG metabolite in gliomas using MRS, including the therapeutic potentials of targeting *IDH* mutant cells. The potential translational values of MRS-based IDH detection and future directions are discussed.

MRS Principles

MRS can map metabolic profiles and dynamics *in vivo* or within tissue extracts or intact tissue samples in a laboratory setting. MRS detects MR signals from stimulated nuclear spins in a strong static magnetic field. When placed in an external strong magnetic field (B_0), atomic nuclei with a magnetic dipole moment (such as ^{1}H, ^{13}C, or ^{31}P) precess around B_0 at specific frequencies. By applying an excitation radiofrequency (RF) pulse at resonance with this precession, nuclei can be brought to a higher energy state. Following the excitation, the nuclei recover to the equilibrium state and the absorbed energies are released, emitting RF signals. The RF receiver coils are tuned to detect only the RF signals originating from the excited nuclei. Different nuclei of the same type, e.g., ^{1}H, experience slightly different magnetic fields with respect to their molecular environment and therefore precess at slightly different frequencies. The frequency of detected signals is described on a field-independent dimensionless scale called chemical shift (δ), which is expressed in parts per million (ppm; see [16]). Individual molecular properties will be characterized by a single resonance (e.g., *N*-acetylaspartic acid) or multiple resonances (e.g., lactate, a doublet resonance) in the MR spectra (Figure 2*A*). The signal intensity reflects the number of excited nuclei in the molecule. The MRS technique enables detection of a wide array of metabolites simultaneously. In this review, we refer to studies observing the nucleus of the hydrogen atom (proton, ^{1}H), which is most commonly used in MRS due to its high intrinsic sensitivity.

Ex Vivo *MRS of Biopsy Specimens*

High-resolution magic angle spinning (HRMAS) is a relatively new solid-state MRS technique for metabolic profiling of intact tissue samples. In solid state, molecules experience significant motion restriction. The major reasons are molecular dipole-dipole interactions and chemical shift anisotropy, resulting in spectral broadening with an angular dependency. By rapidly spinning (typically 5000 Hz) the sample at an angle (θ = 54.7°) to the main magnetic field, the so-called "magic angle," the line broadening is markedly reduced, and the tissue samples thus resemble a semiliquid phase [17]. The sample preparation

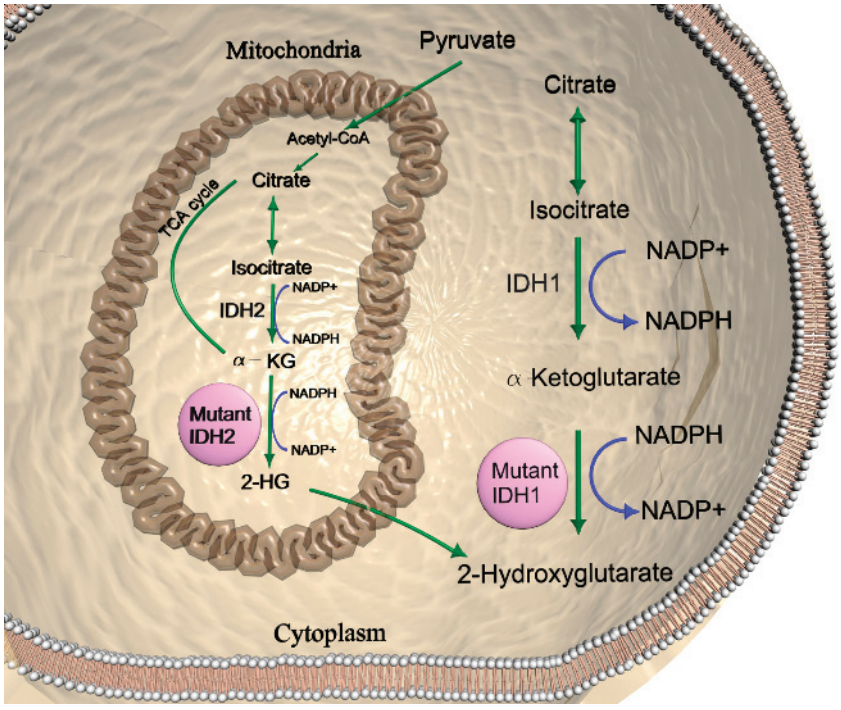

Figure 1. Mutant IDH1/2 causes accumulation of the oncometabolite 2-HG. Metabolic pathway of 2-HG production follows the catalytic reduction of α-ketoglutarate by IDH1 (cytoplasmic) and IDH2 (mitochondrial) through the conversion of NADPH to NADP+.

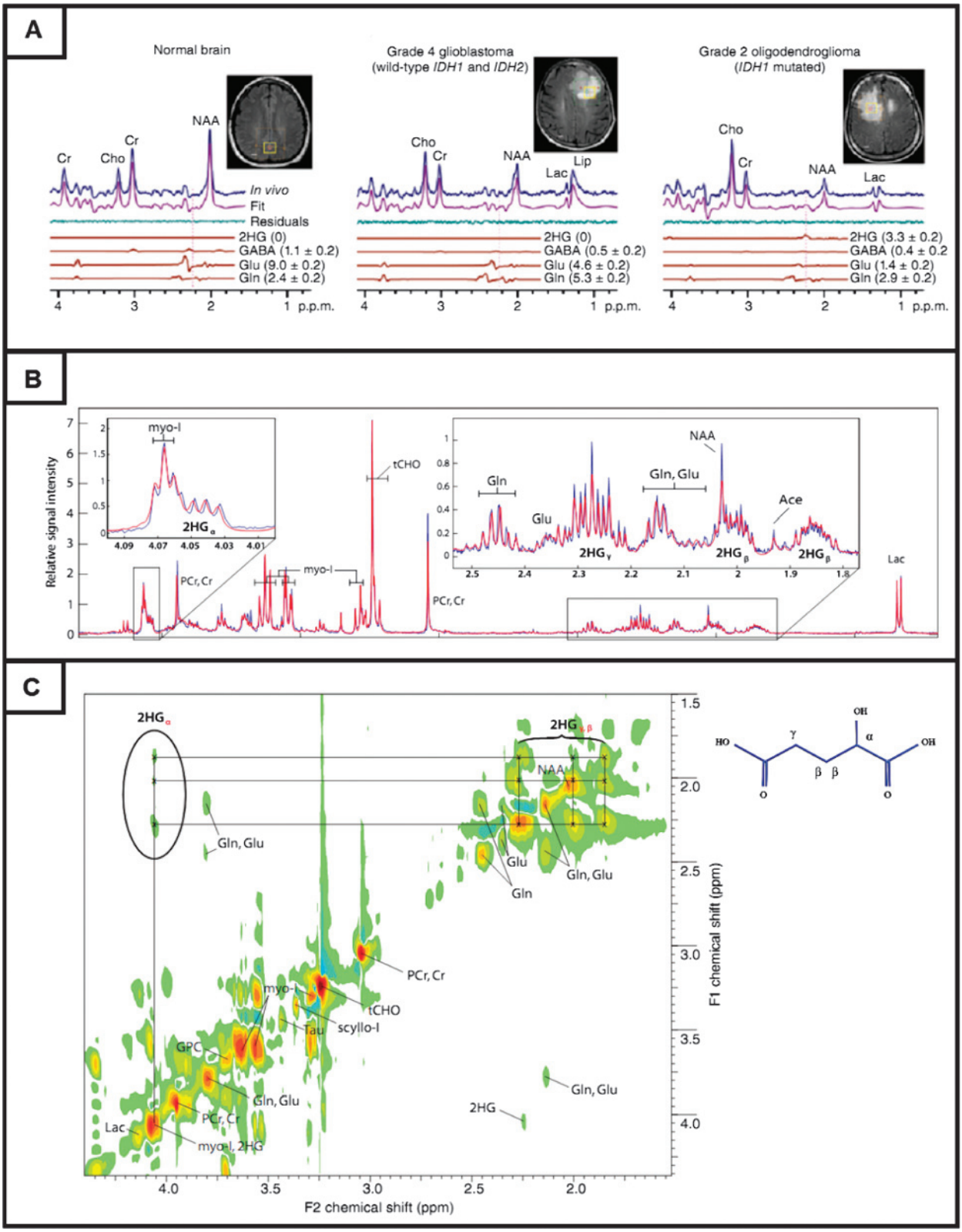

Figure 2. 2-HG detection in *IDH* mutant gliomas and its MRS signature in ^{1}H MR spectra. (A) Representative *in vivo* ^{1}H MR spectra from normal brain and gliomas. Single voxel ($2 \times 2 \times 2$ cm^3)–localized PRESS spectra at 3 T together with spectral fits to the components of 2-HG, γ-aminobutyric acid, glutamate, and glutamine are shown. Vertical lines are drawn at 2.25 ppm to indicate the H_β multiplet of 2-HG in the PRESS-edited spectra. (B) HRMAS spectra from surgical specimens *ex vivo* obtained from glioma sample of WHO grade III astrocytoma; blue and red traces represent the acquired and fitted spectra, respectively. (C) A 2D spectrum acquired from the same tissue sample representing the resonance cross-peaks about the F1/F2 diagonal. Three well-resolved proton resonances of 2-HG are located along a vertical column (cross-peaks of 2-HG$_\alpha$, 2-HG$_\beta$, and 2-HG$_\gamma$) at F2 = 4.05/F1 = 1.85 ppm, 4.05/2.01 ppm, and 4.05/2.28 ppm. Adapted with permission from [14,27].

is simple and straightforward. It typically includes weighing the samples (approximately 5–30 mg), thawing them on an ice bed, and cutting tissue samples either for direct loading into the MAS zirconium rotors (small holders with air turbine to spin the sample) or for loading into disposable inserts fitting the MAS rotors. A small amount of suitable buffer (<5 μl) is used for chemical shift referencing and field locking [18,19]. HRMAS is nondestructive, enabling subsequent biologic evaluations such as histopathology or gene expression profiling in the same tissue sample. This technique is well suited for the examination of unprocessed surgical specimens *ex vivo* in a laboratory setting and has the potential to be implemented in clinical workflows.

In Vivo *MRS*

When used *in vivo*, spatially localized MRS or MR spectroscopic imaging is combined with conventional MR imaging to allow the investigation of metabolic distribution within determined anatomic regions. The MR spectra obtained using clinical scanners suffer from poorer signal-to-noise ratio and spectral resolution compared to *ex vivo* MRS. However, recent advances in MR technology, including access to higher magnetic field strengths, have boosted the clinical potential of *in vivo* MRS. *In vivo* MRS detection of 2-HG in *IDH*-mutated glioma patients holds promise for reliable identification of this metabolite. Using a common spectroscopy sequence and field strength of 3 T [14], which is commonly used in the clinical setting, the 2-HG resonances have been identified without need for more specialized instrumentations. The data acquisition and post-processing methods used in these studies could be also implemented on standard hardware already in place in many MR imaging centers. Thus, the significance of 2-HG detection could be assessed with well-defined clinical settings and larger cohorts.

Detection of 2-HG by MRS

Detection of the 2-HG Metabolite Ex Vivo

HRMAS MRS provides a vast amount of biochemical information from intact tissue samples with minimal sample preparation [18,20]. Many studies have demonstrated the ability of this method to investigate and quantify malignancy-associated metabolites in diagnosis, prognosis, and treatment monitoring in cancer [21–25]. Using HRMAS MRS, the oncometabolite 2-HG has been detected in tissue specimens resected from glioma patients harboring *IDH1/IDH2* mutations *ex vivo* [26–28]. The MR signal arising from the 2-HG metabolite exhibits complex features and overlaps with neighborhood resonances, such as myo-inositol, glutamine, glutamate, and γ-aminobutyric acid (Figure 2*B*). By using two-dimensional (2D) correlation spectroscopy (COSY) [29], the distinctive metabolite cross-peak pattern from the proton correlations in a molecule can be distinguished (Figure 2*C*).

In a study by Elkhaled et al. [27], there was a significant correlation between the presence of 2-HG as determined by MRS and *IDH1* mutation status as determined by IDH1-R132H immunostaining and direct genetic sequencing of *IDH1*. The authors found a strong correlation between 2-HG levels and the presence of *IDH* mutation. They also correlated the 2-HG levels with histopathologic parameters, including cell density in tumor. Kalinina et al. [28] investigated whether the presence of 2-HG in glioma cohorts was reflected as a consequence of *IDH1/IDH2* mutations across the subtypes and grades including nontumorous controls. Beyond the complexity, the 2D MR spectra clearly demonstrated the resolved proton resonances of 2-HG. High sensitivity, specificity, and accuracy (>95%) for the MRS-based identification of *IDH1/IDH2* mutant gliomas with high levels of 2-HG were obtained.

In Vivo *MRS of Glioma Patients*

The feasibility of using 2-HG as a biomarker of *IDH* mutation in gliomas has been further established *in vivo* by using both 1D spectral editing and 2D MRS techniques [14,15]. Using an edited point resolved spectroscopy sequence (PRESS) [30], Choi et al. [14] identified the 2-HG resonances in ^{1}H MR spectra of *IDH*-mutated gliomas at clinical field strength (3 T; Figure 2*A*). In an investigation of glioma patients harboring *IDH1* mutations, primary glioblastoma multiforme (GBM) patients lacking *IDH* mutations, and healthy volunteers, Andronesi et al. [15] found a correlation between the 2-HG accumulation and *IDH*-mutated gliomas (Figure 3). Among 30 patients, the 2-HG resonances were detected only in the ^{1}H MR spectra of *IDH1*-mutated gliomas, not in the GBM and healthy control cohorts, confirming the high specificity [23] of the method. The feasibility of detecting 2-HG using a localized 2D MRS sequence [31] using a 3-T clinical scanner was also proven [15]. The 2-HG metabolites were also assigned in the ^{1}H MR spectra using localized 1D MRS.

Pope et al. [32] found the presence of 2-HG (with a sensitivity of 100%) in low-grade and recurrent GBM with *IDH1* mutation. A conventional single-voxel MRS sequence was used in this study, showing the feasibility of detecting 2-HG resonances with current clinical uses. Analyzing the MR spectra obtained from two cohorts of patients, they found a significantly higher 2-HG level in mutant *IDH1* genotypes than in wild type. The metabolite peaks were fitted using the LC Model software package [33] for estimating the metabolite level of 2-HG. When the glutamine and glutamate levels were compared *ex vivo*, no significant differences were found between wild-type and *IDH1* mutant subgroups. Thus, the 2-HG peaks contributed predominantly to the increases in the detected MRS signals *in vivo* of glutamate + glutamine + 2-HG based on the LC Model measurements [32]. However, the *in vivo* assignment of 2-HG resonances is an important technical challenge because of the complex spin-coupling features. This could lead to false-positive 2-HG detection in wild-type cohorts [32]. The MRS sequences may still need further optimization and refinement to achieve reproducible sensitivity and specificity applicable in clinical settings. In addition, it is important to validate *in vivo* proof-of-principle data by implementing the protocols in a larger cohort.

One of the challenges in studying the underlying biology of *IDH* mutant gliomas *in vivo* could be the lack of reliable preclinical models that can also be used to develop novel therapeutic strategies targeting the *IDH* mutant cells. For this purpose, Luchman et al. [34] established an orthotopic anaplastic oligoastrocytoma xenograft model with endogenous *IDH1* mutations.

Clinical Perspective

More than 70% of low-grade glioma patients (grade II or III) carry *IDH1/IDH2* mutations. Most of the grade II and III gliomas progress over time to glioblastomas and are called secondary glioblastomas. The frequency of *IDH* mutations is high (>70%) among secondary glioblastomas compared to primary glioblastomas (<10%). (Table 1). Thus, 2-HG may potentially distinguish primary glioblastomas from secondary glioblastomas [10,35,36]. Moreover, *IDH* mutations provide overall survival prediction, irrespective of glioma grade. A better prognosis has been generally reported in glioma patients carrying an *IDH* mutation [37,38]. Furthermore, a reduction in cell

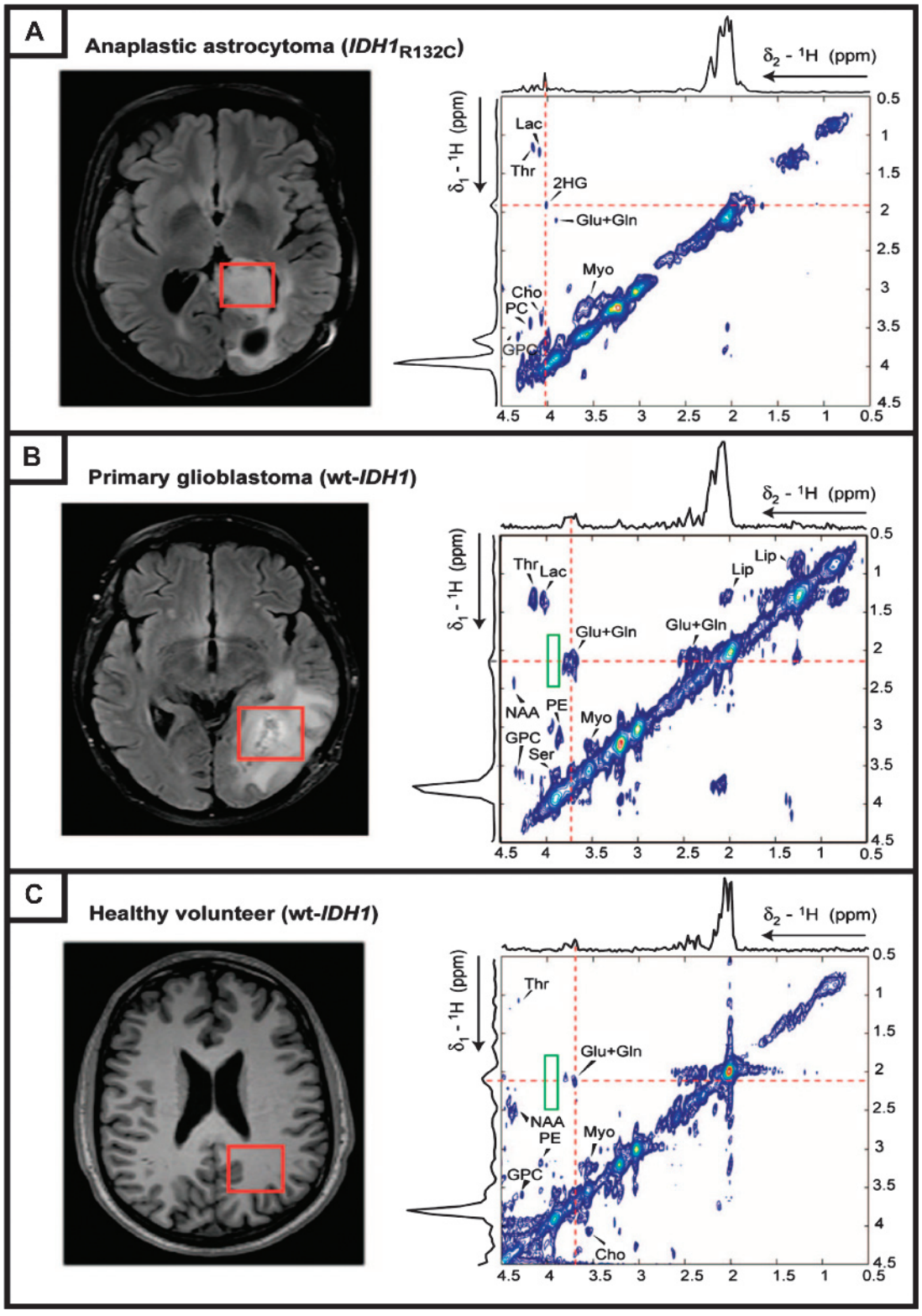

Figure 3. *In vivo* 2D MRS spectra of human brain subjects acquired at 3 T. Single voxels (red rectangles, $3 \times 3 \times 3$ cm^3) are placed on the basis of fluid attenuated inversion recovery images prescribing abnormalities in tumor patients (left column), and the corresponding metabolite cross-peak are depicted as contour maps (right column). (A) Astrocytoma patient with *IDH1*-R132C; the H_α-H_β cross-peak of 2-HG located at 4.02/1.91 ppm (δ_2/δ_1). The 2D spectra acquired (voxel size of $3.5 \times 3.5 \times 3.5$ cm^3) from a primary glioblastoma patient with wt-*IDH1* (B) and healthy volunteer with wt-*IDH1* (C) do not contain any 2-HG cross-peak (outlined by the green rectangle). All 2D spectra were acquired using a developed 2D localized adiabatic selective refocusing–COSY sequence with a repetition time of 45 milliseconds, 64 increments in F1 direction, 8 averages per F1 transient, and a total acquisition time of 12.8 minutes. Adiabatic pulses improve the sequence performance by providing sharp and uniform excitation slices and a robust flip angle and by significantly decreasing the chemical shift displacement error (see [31]). Adapted with permission from [15].

proliferation, as a result of 2-HG accumulation in glioma cell lines, and prolonged survival in mice injected with *IDH1*-R132H mutant cells have been found [39]. Bralten et al. concluded that *IDH1*-R132H mutations in gliomas are associated with better prognosis and reduced aggressiveness, regarding both *in vitro* and *in vivo* results. Translating noninvasive detection of 2-HG by MRS to clinical applications may have a favorable impact on diagnosis, prognosis, treatment stratification, and management of glioma patients.

Summary and Conclusion

Using MRS, the oncometabolite 2-HG can be detected with high sensitivity (>90%) and specificity in gliomas harboring *IDH* mutations *ex vivo* and *in vivo*. The capability of this technique in tracking a mutation event suggests that 2-HG is a clinical valuable biomarker. Thus, it may potentially help to investigate how the *IDH* mutation accompanies gliomagenesis. *Ex vivo* analysis using HRMAS MRS provides a nondestructive and highly sensitive method in which tissue samples can be used for further histopathology and genomic analyses, aiding a better understanding of tumor biology. *In vivo* studies demonstrated the feasibility of detecting 2-HG noninvasively using current clinical scanners. Further validation in larger cohorts may improve the molecular characterization of *IDH*-mutant tumors. In conclusion, MRS is a useful and promising method for analyzing cancer-associated metabolic alteration, providing mechanistic insights into tumorigenesis, and presents a potential for therapeutic intervention.

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