

Metabolic characterization of glioma populations with emphasis on onco-metabolite 2-hydroxyglutarate

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Introduction: A high-profile study on low grade gliomas has recently established a link between mutations in the isocitrate dehydrogenase-1 (IDH1) gene and excessive production of the onco-metabolite 2-hydroxyglutarate (2HG)¹. This is an important finding because overwhelming clinical evidence now suggests that glioma patients harboring IDH1 mutations carry a significant survival advantage, irrespective of treatment therapies employed for disease management^{2,3}. Given the potential prognostic value of metabolites such as 2HG, the goal of this study was to characterize differences in metabolite levels between gliomas of varying origin and grade using proton high-resolution magic-angle-spinning (¹H HR-MAS) spectroscopy.

Methods: Fifty-three patients with recurrent WHO grade 2 glioma (LGG) and twenty-eight patients with newly diagnosed WHO grade 4 glioma (primary GBM) received a pre-surgical MR examination on a 3T GE scanner. Data acquired included 3D lactate-edited ¹H spectroscopy (MRSI), 6-direction diffusion-weighted imaging (DWI), and perfusion-weighted imaging. Automated algorithms generated maps of the choline-to-N-acetyl-aspartate index (CNI), apparent diffusion coefficient (ADC) and perfusion curves.

Image-Guided Tissue Samples: Regions of suspected tumor were identified as having elevated CNI values, low ADC values, or elevated perfusion peak height/reduced recovery and were designated as targets for tissue sampling using surgical navigation software (BrainLab). Tissue samples removed during surgery were divided into two parts and flash-frozen in liquid nitrogen. A pathologist scored one part of the sample for tumor cellularity (*I-III*, in order of increasing cell density) and evaluated histology. The second part of the sample was analyzed with ¹H HR-MAS.

Ex-vivo ¹H HR-MAS: Tissue samples (~5-20mg) were loaded into a 35μL Varian zirconium rotor with 3μL 99.9% atom-D deuterium oxide containing 0.75 wt% 3-(Trimethylsilyl)propionic acid (TSP) prepared by Sigma Aldrich. ¹H HR-MAS spectroscopy was performed at 11.7 Tesla, 1° C, 2250Hz spin rate in a 4mm gHz nanoprobe using a 500MHz Varian INOVA spectrometer. A 1D Carr-Purcell-Meiboom-Gill (CPMG) sequence was acquired with TR/TE=4s/144ms, 512 scans, 40,000 points, 90° pulse angle, and 20,000Hz spectral width. The Electronic Reference To access In-vivo Concentrations (ERETIC) method provided an external standard for quantification⁴. Acquisition of 2D Total Correlation Spectroscopy (TOCSY) followed previously optimized methods for separating choline moieties⁵.

Analysis: The Java-based Magnetic Resonance User Interface (jMRUI) permitted pre-processing of ex vivo spectra in the time domain. A semi-parametric fitting algorithm, High-Resolution Quantum Estimation (HR-QUEST)⁶, was used to quantify 26 metabolites from known concentrations of stock solutions. Analysis was performed on pathologically confirmed tumor samples whose metabolites were fit with <11% Cramer-Rao error bounds. The Wilcoxon ranked-sum test enabled statistical comparisons of patients' mean sample concentrations; statistical significance was defined as p<0.05.

Results:

Patients were classified for analysis according to whether 2HG detection was possible from CPMG spectra (2HG⁺/2HG⁻). 2D TOCSY data often provided further confirmation of 2HG presence, based on the resolution of signature cross-peaks (Fig.1). For the 53 recurrent LGGs, pathology had revealed that 22 remained grade 2, while 25 upgraded to grade 3 and 6 upgraded to grade 4 (secondary GBM). Figure 2 depicts the comparison of 2HG⁺ and 2HG⁻ spectra from grade 2 samples. As shown schematically in Figure 3, there were significant differences, not only in levels of 2HG but also in levels of a large number of other metabolites, among lesions of various grade and origin. These differences are summarized as follows:

- 1) 80% of the 110 recurrent LGG tissue samples showed detectable levels of 2HG but none of the 43 primary GBM tissue samples showed 2HG.
- 2) Of the lesions that remained grade 2 at recurrence, 12 were 2HG⁺, and 4 were 2HG⁻
- 3) PC, GPC, tCho, Gly, Gln, Asp and Lac were higher in 2HG⁺ relative to 2HG⁻ grade 2 lesions
- 4) All of the lesions that had upgraded to grade 3 were 2HG⁺ and 5 out of 6 lesions that had upgraded to grade 4 were 2HG⁺
- 5) All choline metabolites, Glu, Gln, GSH, MI, Tau and betaine were higher in the lesions that had transformed to grade 3 relative to those that remained grade 2.
- 6) Similar changes were seen in the lesions that had transformed to secondary GBM, but in this case PC was the only component corresponding to tCho that was higher.
- 7) Secondary GBM, compared to their primary counterparts, showed elevated levels of 2HG, PC, Cho, MI, Gly, GSH and Lac.

Conclusions: The incidence of 2HG in upgraded glioma precluded casual comparisons between wild-type and mutant IDH varieties, however 2HG⁺ grade 2 glioma suggest that mutant IDH sub-types possess a distinctive metabolic profile. When examining recurrent LGG as a whole, PC, GSH, and betaine are seen as common denominators in the conversion to higher grade. Previous studies have shown that elevated levels of tCho are associated with higher tumor grade and poor prognosis. The results of these ex vivo analyses indicate that while all three components of the tCho peak (Cho, GPC and PC) increase from grade 2 to 3, transformation to secondary GBM is primarily related to an increase in PC. Differences in metabolite levels observed between primary and secondary GBM are of interest in terms of understanding the disparate clinical outcomes of these two populations and distinguishing their diagnoses. 2HG appears to offer a marker for secondary GBM, which, according to theory, are occasionally mis-diagnosed as de novo grade 4, owing to delayed manifestation. Several of these findings are not only relevant for discerning GBM origin or predicting grade transformation, as they may also hold prognostic value and aid in predicting response to therapy.

References: [1] Dang et al. *Nature* 2010;465(7300):966. [2] Yan et al. *N Engl J Med* 2009; 360:765-773 [3] Parsons et al. *Science* 2009;321:1807-12. [4] Albers et al. *Magn Reson Med* 2009;61(3): 525-32. [5] Swanson et al. *Magn Reson Med* 2008;60(1):33-40 [6] Stefan et al. *Meas.Sci.Technol* 2009;20(10)

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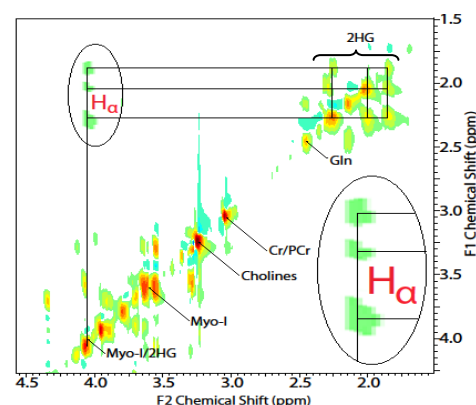


Figure 1: 2D TOCSY spectra of 2HG in a WHO grade 3 astrocytoma; resonance of the alpha hydrogen enhanced

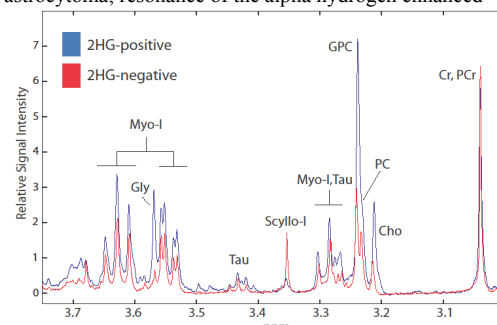


Figure 2: Comparison of metabolite levels in 2HG⁺/2HG⁻ WHO grade 2 samples (spectra normalized by eretic peak area and sample weight)

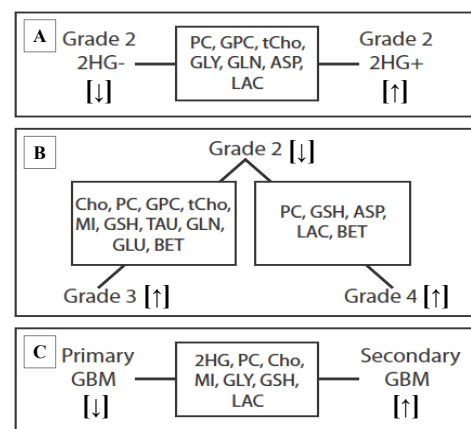


Figure 3: Statistically significant metabolic comparisons: (A) 2HG⁺ grade 2; (B) grade 2 upgrading to grades 3 & 4; (C) primary versus secondary GBM (grade 4)