Image-guided metabolomic analysis of 2-hydroxyglutarate in IDH-mutant gliomas

Llewellyn E. Jalbert[1], Adam Elkhaled[2], Joanna J. Phillips[3], Susan M. Chang[4], and Sarah J. Nelson[1,2]

[1]Bioengineering & Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, United States, [2]Radiology & Biomedical Imaging, University of California, San Francisco, San Francisco, CA, United States, [3]Pathology, University of California, San Francisco, San Francisco, CA, United States, [4]Neurological Surgery, University of California, San Francisco, San Francisco, CA, United States

Introduction: Missense mutations in the isocitrate dehydrogenase 1 and 2 (*IDH1/2*) oncogenes are a common histological feature of gliomas arising from low-grade lineages, present in upwards of 70% of cases[1]. *IDH1/2* encodes the family of isocitrate dehydrogenase enzymes involved in the enzymatic decarboxylation of isocitrate to alpha-ketoglutarate in the citric acid cycle. Curiously, mutations in *IDH1/2* result in the neomorphic gain-of-function ability to produce D-2-hydroxyglutarate (2HG), an oncometabolite that has been implicated in the disruption of numerous cellular processes[2,3]. Furthermore, the presence of *IDH1/2* mutations in patients has been associated with a significant survival advantage and increased sensitivity to radiation and alkylating chemotherapy[4,5]. 2HG has proved to be a promising biomarker that can be quantified using magnetic resonance[6], however little research has been conducted on the affect on 2HG on the cellular metabolome. The goal of this study was to investigate the differences in metabolic profiles between *IDH*-mutant recurrent low-grade gliomas containing 2HG with wild-type *IDH* tumors using proton High-Resolution Magic Angle Spinning (^{1}H HR-MAS) spectroscopy.

Methods: 110 patients with an original pathologically confirmed diagnosis of WHO Grade 2 glioma were included in our IRB-approved study. Patients were recruited immediately prior to resection for suspected recurrence, when progression to higher grade is often observed. They had received prior treatment with surgical resection and/or radiation and chemotherapy.

In vivo MR Scans: Preoperative MR studies were conducted at either 1.5 or 3 Tesla. The scans included standard anatomic imaging, as well as 6 directional axial Diffusion Weighted Imaging (DWI) with b=1000s/mm^2; lactate-edited 3D MRSI with PRESS volume localization; and dynamic Perfusion Weighted Imaging (PWI) with a 5ml/s injection of 0.1mmol/kg body weight Gd-DTPA.

Tissue Acquisition: 1-4 issue sample locations per patient were selected in the BrainLab navigation software based on surgically accessible areas with low ADC, elevated Choline/N-Acetylaspartate index (CNI), or elevated PWI peak height and low recovery. Upon surgical excision, tissue samples were immediately bisected: one half was snap frozen in liquid nitrogen and stored at -80°C for ^{1}H HR-MAS spectroscopy; the other was routinely fixed in 4% formalin, dehydrated by graded ethanols, and embedded in wax using standardized techniques for histopathology analysis and *IDH1* immunohistochemistry using an anti-IDH1R132H antibody. 242 images guided tissues samples were acquired.

Ex vivo ^{1}H HR-MAS: Tissue samples were placed in a 35 μl zirconium rotor custom designed by Varian with 3 μl 99.9% atom-D deuterium oxide containing .75 wt% 3-(Trimethylsilyl)propionic acid (TSP) reference. Samples were scanned at 11.7 Tesla, 1° C, 2250Hz spin rate in a 4mm gHZ nanoprobe with a Varian INOVA 500 MHz multi-nuclear spectrometer. A 1D Carr-Purcell-Meiboom-Gill (CPMG) Sequence was run with TR/TE=4s/144ms, 512 scans, 40,000 acquired points, 90° pulse angle, 20000Hz spectral width, with an acquisition time of 35 minutes. To estimate In-vivo Concentrations, the ERETIC method was utilized for quantification[7]. Levels of metabolites were evaluated using a customized High Resolution Quantum Estimation (HR-QUEST) semi-parametric algorithm, with a basis set that included a spectrum of 2HG, as well as standard metabolites known to be present in brain tissue[8]. HR-QUEST Parameter fits with less than 11% Cramer-Rao error estimates were included for analysis. An experienced spectroscopist classified each spectrum for the presence or absence of 2HG (2HG+/-) and metabolite differences were evaluated at a tissue sample level using a Wilcoxon rank sum test to assess statistical significance ($p<0.05$).

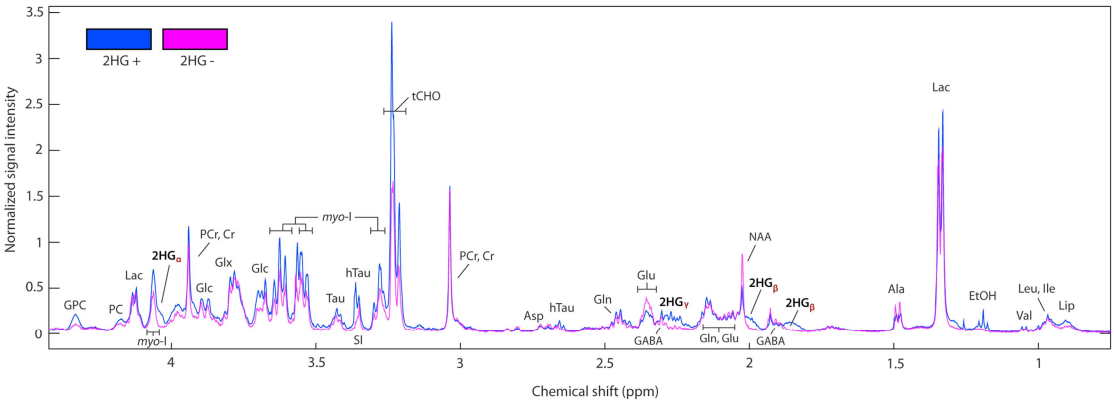

Figure 1. 2HG+ tissue samples display altered metabolic activity. Normalized mean spectra for 2HG+ tissue samples (blue) contained increased levels of many prominent brain tumor metabolites.

Results: Thus far 156 samples from 77 patients have been analyzed using ^{1}H HR-MAS. 25 samples were excluded due to insufficient tissue size and 44 tissue samples were excluded from the 2HG +/- comparison due to insufficient signal-to-noise to unequivocally discern 2HG from neighboring metabolites. This left 71 samples that were classified as 2HG+ and 16 as 2HG-. Wilcoxon rank sum analysis revealed increased levels of metabolites in 2HG+ spectra. These included tCho ($p<0.001$), Bet ($p=0.03$), Gln ($p=0.003$), Glu ($p=0.048$), NAA ($p=0.01$), GSH ($p=0.004$), PE ($p=0.015$), GPC ($p=0.006$), PC ($p=0.01$), Cho ($p=0.01$), and the Myo-I-to-tCho ratio (MCI, $p=0.01$). Differences in histological parameters between the two cohorts indicated increased mitosis (KI-67, $p=0.001$), increased axonal disruption (SMI31, $p=0.001$), and increased ratio of tumor cells / normal cells ($p=0.01$) in 2HG+ tissue samples compared to 2HG-. Within each cohort, spectra were registered and normalized by the ERETIC signal and sample mass, then averaged to create a distinct spectral profile for 2HG+ and 2HG- tissue samples (Figure 1).

Discussion: The ultimate goal of this project is to improve the clinical management of patients with recurrent low-grade gliomas. This information may aid in elucidating the differences in metabolism between lesions that have IDH mutations and those that do not. Increased metabolite levels and histopathology parameters associated with malignancy in 2HG+ tumors support the altered metabolic state of *IDH*-mutant tumors and suggest that this may be related to increased cellularity and mitotic activity in these lesions. Future analysis will be focused on analysis at the patient level and correlations with *in vivo* parameters from the pre-surgical MRI.

References: [1] Yan, et al. (2009) NEJM 360:765-73 [2] Dang, et al. (2009) Nature 462:739-743 [3] Shih, et al. (2012) Cancer Cell 22:285-287 [4]Li, et al. (2012) Neuro-Oncology [5] Houillier, et al. (2010) Neurology 75:1560-1566 [6] Elkhaled, Jalbert, et al. (2012) Sci. Transl. Med. 4,116ra5 [7] Albers et al. (2009) Mag. Reson. Med. 61(3):525-32 [8] Ratiney, et al. (2009) NMR Biomed. 23:1-13 *This work was supported by the NIH Brain Tumor SPORE Grant P50CA097257*