COMPARATIVE ^{1}H-MRS STUDY OF IDH1 AND IDH2 MUTATED GLIOMAS IN RODENT BRAIN AT 9.4T

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Target audience: The target audience for this work is clinicians and scientists who are interested in in vivo metabolic profiling in gliomas.

Introduction: Somatic mutations of the isocitrate dehydrogenases 1 and 2 genes (*IDH1* and *IDH2*) in gliomas result in a high accumulation of the onco-metabolite 2-hydroxyglutarate (2-HG) [1,2]. Detection of 2-HG in human patients by using ^{1}H-MRS has been reported in previous pioneering studies [3,4] where spectral editing methods with long echo times (TEs) had to be employed due to the severe spectral overlaps and low SNR at clinical field strength. While the feasibility of detecting the target signal from 2-HG in vivo was clearly demonstrated in these studies, precise quantification of other metabolites may be difficult as they were partially or even almost completely suppressed in the edited spectra, thereby potentially hindering a more comprehensive *in vivo* metabolic profiling of the IDH-mutated gliomas. Given the increasing availability of high-field clinical scanners, therefore, we have evaluated the feasibility of assessing IDH-mutational status and metabolic profiling *in vivo* at 9.4T with a minimal TE in rodent brains with IDH-mutated gliomas. In this experimental setting, signal for all metabolites are equally maximal, and this may potentially be beneficial for a more comprehensive metabolic profiling of the IDH-mutated gliomas.

Methods: The study was approved by IACUC. Six-week-old Fisher 344 female rats (F98 IDH1 R132H, n=13; F98 IDH2 R172K, n=10) with orthotopic brain tumors with IDH1/2-mutation were used. For induction of the brain tumors, IDH1/2MT-F98 rat glioma cells were transplanted into the right striatum. MRS studies were conducted on a 9.4T MR scanner (Agilent) at 2 weeks after the F98 gliomas implantation. Prior to MRS data collection, T_2-weighted scout images were acquired. Using a SPECIAL sequence [5] (TR/TE = 4000/2.83 ms, spectral BW = 5 kHz, 2048 data points zero-filled to 4096, and 384 averages), MRS data were collected from a tumor region and a (contralateral) normal region of the brain for each animal. Therefore, the total numbers of voxels for IDH1 R132H, IDH2 R172K and normal brain regions were 13, 10, and 23 respectively. Voxel volumes ranged 7~15 µl. In order to account for potential differences in spectral baseline among the animals, metabolite-nulled baseline spectra were also acquired for all animals with a SPECIAL sequence modified for double inversion [6] (HS10 pulses, BW = 6.6 KHz, duration = 3 ms). The sequence parameters were: TI_1(1st inversion time)/TI_2/TR = 2150/680/4650 ms and 320 averages. The rest of the sequence parameters were identical to those used for the metabolite quantification. Data were processed by using MRUI [7]. Residual water signal was removed by the HLSVD filter. Based on the metabolite-nulled spectra, baseline spectra were modeled for each voxel for all rats individually, and then included in the basis set for spectral fitting. The QUEST time domain method [8] was used for metabolite quantification. Peaks were normalized to the total creatine (tCr) signal. Finally, quantified data sets were statistically analyzed by using one-way ANOVA and Newman-Keuls multiple post-hoc comparison tests.

Results and Discussion: Representative spectra are shown in ***Figure 1*** along with the T_2-weighted images for a IDH1 R132H tumor region (A), a IDH2 R172K tumor region (B), and a normal brain region (C). The fitted spectra are also shown for several metabolites including 2-HG. No false-positive 2-HG detection occurred for all voxels in normal brain regions. 2-HG was successfully detected for the majority of the IDH1/2-mutated tumor regions (IDH1 R132H: 9/13, IDH2 R172K: 10/10). Those 4 spectra with false negative errors from the IDH1 R132H regions were all severely contaminated by strong lipid signal. Other major metabolites were quantified with high accuracy as indicated by the mean SD% of the fitted results (e.g., SD% 2-HG: 3%, total choline (tCho): 10%, tCr: 9%, lactate (Lac): 3%, N-acetylaspartate (NAA): 5%, taurine (Tau): 3%). The key findings from our quantification results were summarized in ***Figure 2***. The 2-HG/tCr level was upregulated in the IDH2-mutated F98 tumors compared to that of the IDH1-mutated tumors. The Lac/tCr level was decreased in the IDH2-mutated tumors with respect to that of the IDH1-mutated tumors. These results suggest that F98 gliomas bearing somatic mutations of IDH1/2 genes have different metabolic profiles from each other in our animal models *in vivo*. The lower Lac/tCr in F98 IDH2-mutated gliomas may indicate that the Warburg effect [9] was overridden therein with respect to IDH1-mutated gliomas for the energy supply necessary for the higher production of 2-HG. These findings in our study are in line with a previous *in vitro* study with IDH1/2-mutated U87 cells [10]. In summary, our preliminary results support that the *in vivo* assessment of IDH-mutational status in gliomas may be feasible at 9.4T upon, when necessary, effective suppression of lipid signal. The equally maximal signal yield for all metabolites with a minimal TE and subject-specific baseline modelling with a double inversion sequence module at high field may allow for a comprehensive metabolic profiling of IDH-mutated gliomas *in vivo*. Our results also support the feasibility of preclinical animal studies for the development of novel therapeutic strategies and monitoring treatment outcomes for IDH-mutated gliomas *in vivo*.

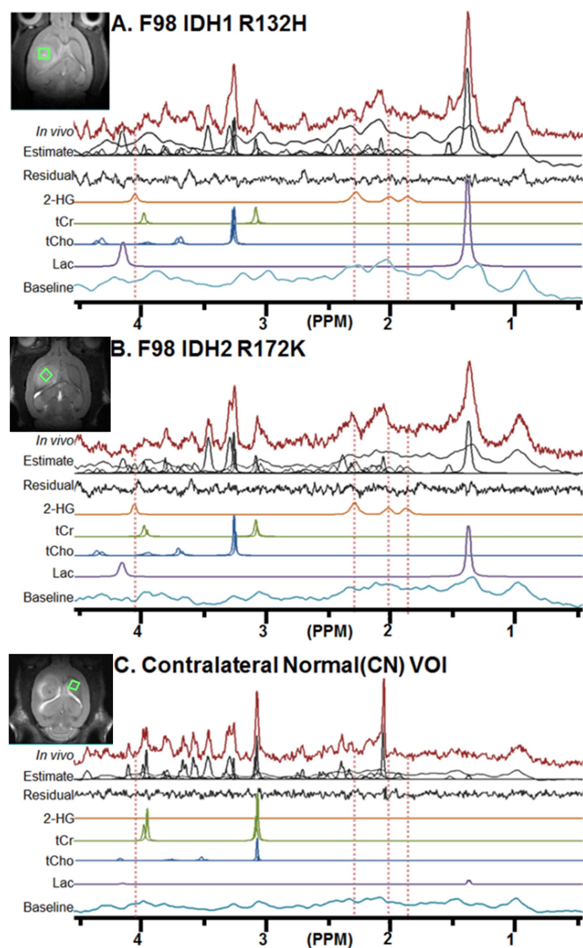

Figure1. Representative ^{1}H-MRS spectra and voxel positions. The fitted results are also shown for several metabolites including 2-HG (A: IDH1-R132H, B: IDH2-R172K, C: Contralateral normal).

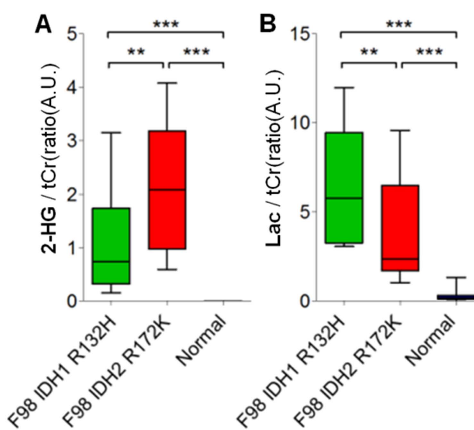

Figure2. Metabolic alterations in IDH1- and IDH2-mutated tumors with respect to contralateral normal brain regions. Peaks were normalized to tCr (One-way ANOVA p<0.0001, Newmann-Keuls comparison test score ** & ***).

Reference [1] Parsons *Science* 2008, [2] Andronesi *JCI* 2013, [3] Choi *Nat Med* 2012, [4] Andronesi *Sci Transl Med* 2012, [5] Mlynarik *MRM* 2006, [6] Cudalbu *J Alzheimers Dis* 2012, [7] Naressi *MAGMA* 2001, [8] Ratiney *MAGMA* 2004, [9] Warburg *Science* 1956, [10] Wen *J Neurochem* 2014.

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