Ex-vivo identification of IDH mutant gliomas using edited magnetic resonance spectroscopy detection of 2HG at 9.4 Tesla

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Introduction. Diffuse gliomas are the most common primary brain tumours in adults. In human gliomas, mutations in the isocitrate dehydrogenase gene (IDH1 and IDH2) are relatively common, and these mutations define a glioma subtype with improved prognosis. Recently, it has been shown that IDH mutated gliomas undergo neomorphic enzymatic activity which leads to an accumulation of 2-hydroxyglutarate within the tumour [1]. Magnetic resonance spectroscopy (MRS) detection and quantification of 2HG therefore represents a potentially powerful tool for the initial staging and management of diffuse gliomas. In this study, twelve glioma biopsy samples were examined ex-vivo using MRS at 9.4 Tesla with the aim of determining whether MRS measurements of 2HG can predict IDH mutation status.

Methods. Twelve tumour specimens and one normal brain specimen were obtained from twelve patients and immediately snap frozen. A small portion of each specimen was retained for the imunohistochemical (IHC) analysis and IDH sequencing. Perchloric acid extraction was performed on the remainder of each specimen to generate an aqueous metabolite solution for MRS analysis. To enable unambiguous detection of 2HG by MRS, a localized J-difference editing scheme was implemented, based on the MEGA-PRESS method [2,3]. In the implementation presented here, the C2 resonance of 2HG at 4.022 ppm was chosen as the observed resonance, and the editing pulse was applied at 1.9 ppm, near the frequency of its coupling partners at C3. Quantum mechanical simulations were performed using an in-house MATLAB based implementation of the density matrix formalism to determine the optimal echo time for MEGA-PRESS detection of 2HG at 9.4 Tesla. All MEGA-PRESS radiofrequency pulses were modeled as instantaneous rotations, and the inversion profiles of the editing pulses were fully modeled to account for the editing flip angle experienced by each spin. The optimal echo time was determined to be 86 ms (results not shown). The 2HG spin system was modeled using the chemical shifts and coupling constants provided in [4]. All MRS experiments were performed on a 160 mm horizontal bore 9.4T Varian (Palo Alto, CA, USA) MRI scanner using a 26 mm inner diameter transmit/receive quadrature birdcage coil. The extracted tissue sample was positioned in the centre of the birdcage resonator and manual shimming was performed to achieve a water linewidth of less than 15 Hz in all samples. Edited MEGA-PRESS spectra were acquired using the following parameters: 8192 spectral points, spectral width = 4.0064 kHz, TR/TE=2000/86 ms, voxel size = 5 x 5 x 10, or 4 x 4 x 4 mm^3 (depending on sample size), 6400 averages (water suppressed) or 8 averages (water unsuppressed). The editing pulse was applied at 1.9 ppm and had a bandwidth of 77 Hz, such that both the C3 (1.825 ppm) and the C3' (1.977 ppm) spins experienced approximately 88% inversion. Spectral analysis was performed using AMARES [5]. 2HG concentration was estimated by comparing the fitted area under the edited C2-2HG peak (4.022 ppm) to the area of the unsuppressed water peak, assuming a water concentration of 55 mM, taking into account the 2HG/H20 proton ratio, and the estimated editing efficiency of the acquisition.

Results and Discussion. Results. MRS, DNA sequencing and IHC data from six of the 12 tumour specimens are shown in Figure 1. Elevated 2HG is observed in the spectra of all IDH mutated specimens (cases 2 through 6), while the spectrum from a wild type specimen (Case 1) showed no detectable 2HG peak. Table 1 summarizes the MRS, DNA sequencing and IHC results from all 12 subjects. Discussion. 2HG was detected in 9 of 11 IDH mutant tumour specimens. Sampling from the probable tumour infiltration zone accounted for the two samples that did not contain detectable 2HG. One case (Case 6) contained 2HG despite a negative IHC result due to the presence of a less common R132C mutation not detectable by the R132H-specific antibody but present in the sequencing electropherogram. It should be noted that due to a number of factors, the absolute concentration of 2HG measured by MRS in tissue extracts may differ from the 2HG concentration in tissue at the time of biopsy. However, MRS still predicts mutation status with reasonable specificity. This technique can be translated to in-vivo studies, and future work will be aimed at the detection of 2HG in IDH mutated gliomas in-vivo.

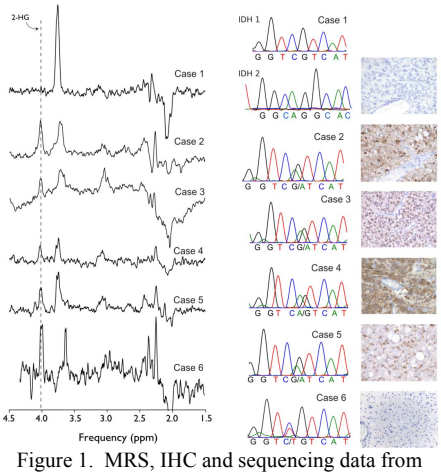

Figure 1. MRS, IHC and sequencing data from six tissue specimens.

Case	MRS [2HG] (mM)	IHC	sequencing
1	0	-ve	W/T
2	29.7	+ve	R132H
3	16.2	+ve	R132H
4	18.8	+ve	R132H
5	13.1	+ve	R132H
6	27.8	-ve	R132C
7	14.7	+ve	R132H
8	0	+ve	R132H
9	7.4	+ve	R132H
10	0	+ve	R132H
11	6.8	+ve	R132H
12	18.8	+ve	R132H
Normal Brain	0	Not done	W/T

Table 1. MRS, IHC and sequencing results from all twelve specimens.

References. [1] Dang L et al. Nature (2009); 462:739-744. [2] Mescher M et al. NMR Biomed (1998); 11:266-272. [3] Choi C et al. Proc Intl Soc Magn Reson Med (2011); 19:304. [4] Bal et al. Magn Reson Chem (2002);40:533-536. [5] Vanhamme L et al. J Magn Reson (1997); 129:35-43.