In vivo assessment of IDH status in glioblastoma using 2D ^{13}C dynamic CSI of hyperpolarized α-ketoglutarate

Myriam Marianne Chaumeil[1], Peder E.Z. Larson[1], Hikari A. I. Yoshihara[1], Daniel B. Vigneron[1], Sarah J. Nelson[1], Russell O Pieper[2], Joanna J Phillips[2], and Sabrina M Ronen[1]

[1]*Radiology, University of California, San Francisco, San Francisco, CA, United States,* [2]*Neurological Surgery, University of California, San Francisco, San, CA, United States*

INTRODUCTION

Mutations in the isocitrate dehydrogenase (IDH) enzyme have been reported in over 70-80% of low grade gliomas and upgraded glioblastomas (GBM)[1,2]. The mutation is an early oncogenic event, and thus mutant IDH inhibitors are under development as novel therapeutic approaches. Non-invasive methods to monitor IDH status *in vivo* are therefore needed for patient stratification and to monitor therapeutic response. Wild-type IDH catalyzes the decarboxylation of isocitrate to α-ketoglutarate (α-KG); in contrast, mutant IDH catalyzes the reduction of α-KG into 2-hydroxyglutarate (2-HG). To date, IDH status has been assessed using ^{1}H magnetic resonance spectroscopy (MRS) methods that detect 2-HG accumulation in biopsy samples and *in vivo* in patients [3-5]. We previously showed the potential of ^{13}C MRS to monitor the metabolism of hyperpolarized (HP) α-KG as an alternative and potentially complementary approach for informing on IDH status in cell lysates and live perfused cells [6]. The goal of this study was to translate these methods to the *in vivo* setting and to evaluate the potential of HP α-KG to assess 2-HG production, and thus IDH status, in orthotopic GBM tumors at clinical field strength.

MATERIAL & METHODS

Tumor-bearing animals and experimental set-up To generate wild-type and mutant IDH tumors, U87 GBM cells were transduced with a lentiviral vector coding, respectively, for wild-type IDH1 (U87IDHwt cells), or for mutant IDH1 and wild-type IDH1 (U87IDHmut cells; modeling the fact that the mutation is heterozygous in patients). Orthotopic tumors were implanted in athymic rats by injecting a suspension of cells (~$3x10^5$ in 10μL) in the right caudate putamen (U87IDHmut, Rats 1-3; U87IDHwt, Rat 4). Experiments were performed on a 3T clinical MR system (GE Healthcare) equipped with a ^{1}H-^{13}C coil (Ø$_I$=80mm). For MR acquisitions, rats were anesthetized using 2% isoflurane in O_2 and a 23G catheter was secured in the tail vein.

Hyperpolarized α-KG 53μL of a [1-^{13}C]-α-KG solution (5.9M, 3:1 water:glycerol, 17.3mM OX63 radical, 0.4mM Dotarem) was polarized using a hypersense polarizer (Oxford Instruments) for 1 hour. After dissolution in Tris-based buffer, HP α-KG (2mL, pH 7.8, final concentration 100mM) was injected through the catheter over 12s.

^{13}C MR acquisition and processing ^{13}C 2D dynamic CSI was acquired starting either 20s (Rats 1 & 2) or 5s (Rat s3 & 4) after the beginning of α-KG injection using two multi-band flip angle schemes: 5° on the α-KG and 30° elsewhere (Rat 1) or 4° on the α-KG and 25° elsewhere (Rats 2, 3 and 4). Matrix 8x18, TE/TR=140/215ms, 5mm resolution, thickness 2cm, image every 5s [7]. ^{13}C datasets were processed using the in-house SIVIC software to calculate the integrals of α-KG, 2-HG and glutamate.

RESULTS & DISCUSSION

Because we first needed to optimize 2-HG detection *in vivo*, each U87IDHmut tumor- bearing animal included in this initial study (Rats 1-3) underwent a slightly different experimental protocol for acquisition of the HP ^{13}C data (see M&M section). Figure 1 summarizes our findings in these 3 rats. MR anatomical images overlaid with the grid used for HP ^{13}C 2D CSI are shown in Figure 1.A (red: tumor, blue: normal brain). Twenty seconds after the start of HP α-KG injection (HP α-KG resonance at 172.9 ppm), a peak at 183.8ppm, which is the expected resonance of HP 2-HG, could be detected in the tumor region in all 3 animals (Fig 1.B). No peak at 183.8ppm could be detected outside of the tumor region. Furthermore, no peak was observed at 183.8ppm in Rat 4, which had a wild-type IDH tumor (data not shown).

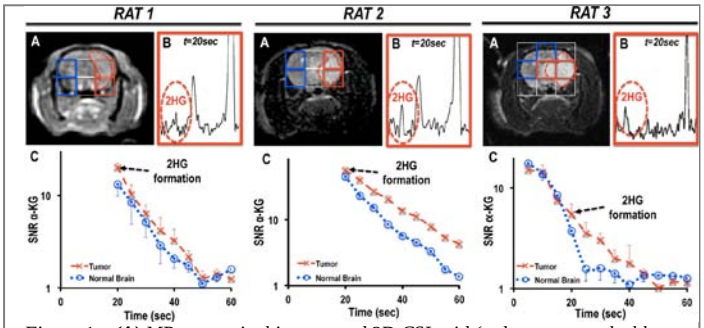

*Figure 1 – **(A)** MR anatomical images and 2D CSI grid (red: tumor voxels, blue: normal brain voxels) **(B)** HP ^{13}C spectrum from tumor voxel showing 2-HG production 20 seconds post beginning of HP α-KG injection **(C)** SNR of α-KG as a function of time (average over tumor voxels in red and brain voxels in blue), showing the higher level of HP α-KG in the tumor region.*

Figure 2 focuses on the data obtained on Rat 3. Fig. 2.B illustrates the high level of HP α-KG in the blood vessels underneath the brain, and the elevated level of HP α-KG in the tumor region (dotted lines) 20s post injection. Importantly, as seen in Fig 2.C, the 2-HG peak was only observed in the tumor region, but not in the area underneath the brain or in the normal brain voxels. Furthermore, 2-HG appeared 20 seconds post injection of HP α-KG in the tumor voxel, and no resonance at 183.8ppm could be observed prior to the 20s time point (Fig. 2.D). Collectively, these data indicate that 2-HG was produced within the tumor, rather than originating elsewhere. Figure 3 shows the data observed in Rat 1. Interestingly, HP glutamate formation at 177.5ppm was also detected in this rat and Rat 4, both inside the tumor and in the surrounding normal brain, 25s post injection of HP α-KG. This indicates that, in some cases, sufficient amounts of HP α-KG might cross the blood brain barrier (BBB) to enable detection of normal metabolism *in situ* (Fig. 3 B.).

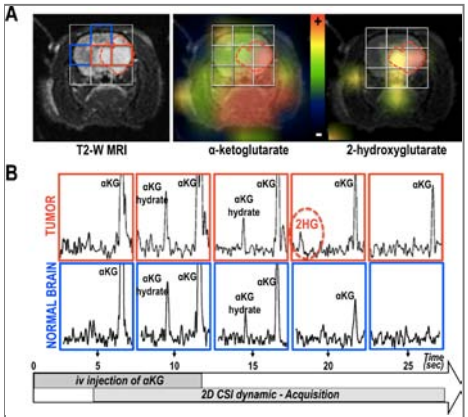

*Figure 2 – **(A)** 2D CSI grid, **(B)** α-KG heatmap, **(C)** 2-HG heatmap at 20 seconds post start of injection. **(D)** Spectra from normal brain (blue) and in tumor (red) and showing 2-HG in tumor at 20s post α-KG injection.*

Interestingly, the signal-to-noise ratio (SNR) of HP α-KG was higher in the tumor region as compared to normal brain in all 3 U87IDHmut animals (Fig 1.C.), and the α-KG signal decreased slower. This suggests higher delivery and retention of HP α-KG, likely due to increased neoangiogenesis, elevated vascular permeability, and BBB breakdown. This effect enhances our ability to detect metabolism of α-KG within the tumor region.

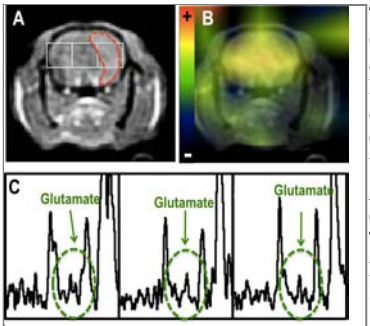

*Figure 3 – **(A)** 2D CSI grid and glutamate heatmap at 25 sec post beginning of injection. **(B)** Corresponding spectra showing glutamate at 177.5ppm*

To conclude, although this study was performed on a limited number of animals, our results indicate that HP α-KG provides a promising agent for interrogation of brain metabolism *in vivo*. α-KG derivatives known to have higher BBB permeability could further enhance this approach. Nonetheless, 2-HG production from HP α-KG can be detected *in vivo* in orthotopic mutant IDH tumors, and can thus be used to distinguish between mutant and wild-type IDH GBMs.

ACKNOWLEDGMENTS Work was supported by Brain Tumor SPORE CA097257, NIH grants R21 CA120010, RO1 CA130819, an American Brain Tumor Association fellowship, and center grant P41EB013598.

REFERENCES 1. Chowdhury, R., *et al. EMBO Rep* 12, 463-469 (2011). 2. Dang, L., *et al. Nature* 462, 739-744 (2009). 3. Andronesi, O.C., *et al. Sci Transl Med* 4, 116ra114 (2012). 4. Choi, C., *et al. Nat Med* (2012). 5. Elkhaled, A., *et al. Sci Transl Med* 4, 116ra115 (2012) 6. Chaumeil, M.M., *et al. ISMRM* 4352 (Melbourne, 2011). 7.Larson, P.E., *et al. J Magn Reson* 194, 121-127 (2008).