Hyperpolarized [1-^{13}C] glutamate: a surrogate marker of IDH1 mutational status in glioblastoma

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INTRODUCTION

Mutations in the isocitrate dehydrogenase (IDH) enzyme have been reported in over 70% of low grade gliomas and upgraded glioblastomas (GBM) [1], are considered an early oncogenic event [2], and are associated with global modulations in the methylome, transcriptome and metabolome [3,4]. Consequently, mutant IDH1 inhibitors are being developed as therapies [5], and non-invasive methods are needed to monitor IDH1 status. Whereas wild-type (wt) IDH1 converts isocitrate to α-ketoglutarate (αKG), mutant IDH1 reduces αKG into 2-hydroxyglutarate (2HG).We recently showed that hyperpolarized (HP) 2HG production from HP αKG could be detected *in vivo* using ^{13}C MRS to non-invasively inform on mutant IDH1 activity [6]. We have also previously reported that it is possible to detect the conversion of HP αKG to HP glutamate in cells [7]. In this study, we expanded on our previous findings and showed that HP glutamate production from αKG could serve as another surrogate marker of IDH1 status (Fig 1).

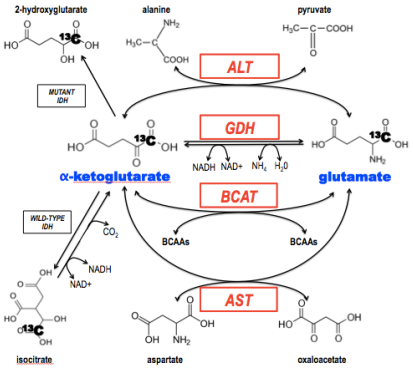

Figure 1 – αKG metabolism, showing the four enzymes catalyzing the αKG to glutamate conversion.

MATERIAL & METHODS

Cell production and culture U87 GBM cells were transduced with a viral vector coding for the wild-type IDH (U87IDHwt) or mutant IDH enzyme (U87IDHmut, R132H variant) [6,7].

Perfused cells Cells were grown on microcarrier beads, (3x10^7 cells, n=5 per cell line); beads were loaded into a 10-mm NMR tube connected to a perfusion system, as previously described [8].

Tumor-bearing animals Orthotopic tumors were implanted in athymic rats (n=2) by injecting a suspension of cells (~3x10^5 in 10μL) in the right caudate [6,9]. A 23G catheter was secured in the tail vein.

Hyperpolarized α-KG [1-^{13}C] αKG solution (5.9M) was polarized using a Hypersense polarizer for 1h and dissolved in buffered solutions. α-KG was then rapidly injected either into perfused cells (concentration 15mM) or intravenously in animals (concentration 100mM).

^{13}C MRS acquisition and analysis Perfused cells: dynamic sets of HP ^{13}C spectra were acquired on a 500-MHz INOVA spectrometer (Agilent Technologies) using 13deg excitation pulses and 3sec TR. *In vivo:* 5sec after the start of iv injection, 2D dynamic CSI ^{13}C data were acquired on a 3T clinical MR system (GE Healthcare) equipped with a ^{1}H-^{13}C coil ($Ø_l$=80mm) using a multi-band variable flip-angle scheme [10] optimized for detection of HP glutamate. Cell ^{13}C spectra were quantified using ACD/Spec Manager and Gamma-variate (GVA) and Monte-Carlo analysis were performed [11]. *In vivo* data were processed using the in-house SIVIC software [6].

mRNA and protein expression were quantified using real-time PCR and western blot analysis, resp., for the four main cytoplasmic isoforms of the enzymes that could catalyze the conversion of αKG to glutamate, namely: alanine transaminase 1 (ALT1), glutamate dehydrogenase 1 (GDH1), branched chain amino-acid transaminase 1 (BCAT1) and aspartate aminotransferase 1 (AST1) (Fig.1).

RESULTS & DISCUSSION

Injection of HP αKG ($\delta_{\alpha\text{-}KG}$=172.9ppm) in U87IDHwt cells resulted in a significant build-up of HP [1-^{13}C] glutamate (δ_{GLU}=177.5ppm, Fig. 2A), which confirmed that sufficient HP αKG permeated the cells to undergo detectable metabolism [7]. Importantly, HP glutamate was barely detectable in U87IDHmut cells (Fig. 2B). As shown in Fig 2C, the area under the curve was decreased by 84.1±5.1% in U87IDHmut cells ($p<0.01$), demonstrating significantly less HP glutamate production.

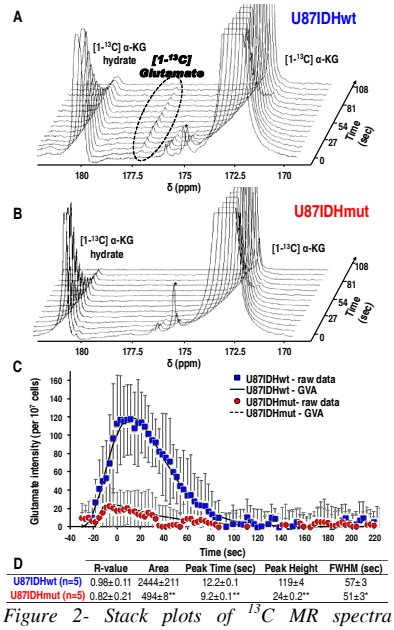

*Figure 2- Stack plots of ^{13}C MR spectra following injection of HP αKG in live U87IDHwt (**A**) and U87IDHmut (**B**) perfused cells (resolution 9sec). (**C**) Intensities of HP glutamate in U87IDHwt (■) and U87IDHmut (○) cells, (D) Results of the GVA of glutamate kinetics (mean±sd; FWHM=full width at half-maximum; student t-test **p<0.005, *p<0.05).*

Next, we wanted to confirm that HP glutamate production was detectable *in vivo*. The 2D CSI grid is overlaid to the MR image for a U87IDHmut and a U87IDHwt animal (Fig. 3A). Heatmaps illustrate high levels of HP αKG in the tumors, regardless of IDH1 status (Fig. 3B). In contrast, HP glutamate was present in U87IDHwt tumor only, in line with the cells results (Fig. 3C). ^{13}C spectra from the tumor voxel (red) show glutamate in U87IDHwt tumors only (SNR_{GLU}=3, Fig 3D). Further studies are underway to confirm these findings in a larger number of animals.

Finally, in order to investigate the potential mechanism for the observed drop in HP glutamate in mutant IDH1 cells, mRNA and protein levels were quantified for the four relevant enzymes (Fig. 1). BCAT1 and AST1 mRNA levels (Fig. 4A) and protein levels (Fig. 4B) were significantly decreased in U87IDHmut cells (*$p<0.01$ for all results). GDH1 and ALT1 mRNA levels and protein levels were unchanged (data not shown). Taken together, these results provide a possible mechanism for the observed drop in HP glutamate, and are in line with a recent report of decreased BCAT1 expression in mutant IDH1 cells [12].

Collectively, our findings indicate that HP glutamate production from HP αKG can be detected in perfused cells and *in vivo* using ^{13}C MRS, and is decreased in U87IDHmut cells/tumors. HP glutamate could thus potentially serve as a non-invasive additional surrogate marker of IDH1 mutational status in brain tumors.

Acknowledgements: NIH R21CA16154, NIH R01CA154915, NIH P41EB013598. **References**: 1. Dang, Nature (2009); 2. Yang, Clin Cancer Res (2012); 3. Huse, Glia (2011); 4. Lu, Nature (2012); 5. Rohle, Science (2013); 6. Chaumeil, Nature Comm (2013); 7. Chaumeil, ISMRM (2011) 8. Ward, Cancer Res (2010); 9. Chaumeil, Neuroimage (2012); 9. Xing, JMR (2013); 10. Lupo, MRM (2010); 11. Tönjes, Nature Med (2013).

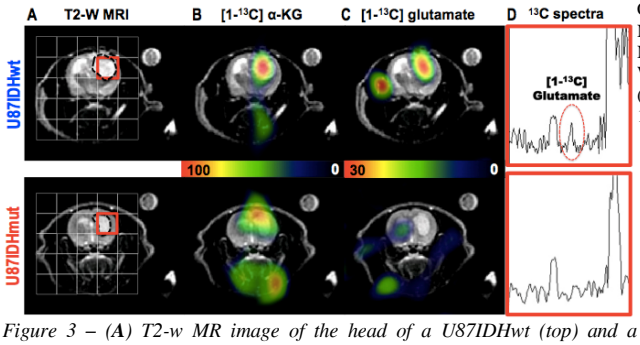

*Figure 3 – (**A**) T2-w MR image of the head of a U87IDHwt (top) and a U87IDHmut (bottom) tumor-bearing animal overlaid with the grid used for 2D ^{13}C CSI acquisition. Corresponding heatmaps of (**B**) HP αKG and (**C**) HP glutamate at 20s post injection, illustrating the presence of this metabolite in IDH1wt tumors only. (**D**) Corresponding ^{13}C spectra from tumor voxels.*

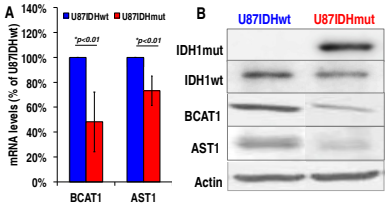

*Figure 4 –BCAT1 and AST1 (**A**) mRNA levels and (**B**) protein levels for U87IDHwt (blue) and U87IDHmut (red) cell lines.*