13C MRS detects TCA down-regulation in mutant IDH1 glioma cells

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Background: Wild-type isocitrate dehydrogenase (IDH) is the enzyme that catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) whereas mutant IDH catalyzes the conversion of α-KG into 2-hydroxyglutarate (2-HG). Mutations in IDH1 have been reported in over 70% of low-grade gliomas and secondary glioblastomas (GBM). These mutations are associated with the accumulation of 2-HG within the tumor and are believed to be one of the most early events in the development of low grade gliomas. Despite these observations, the metabolic fluxes associated with 2-HG production are not fully understood. The goal of this study was to use ^{13}C MRS to probe the fate of ^{13}C-labeled metabolites at thermal polarization and polarized by dynamic nuclear polarization (DNP) and to monitor the glycolytic pathway and the TCA cycle in wild-type and mutant IDH1 glioma cells.

Material and Methods: U87 cells expressing mutant IDH1 (U87IDHmut) and wild-type IDH1 (U87IDHwt) were investigated. Wild-type cells were generated by transduction with a lentiviral vector coding for wild-type IDH1 and mutant cells were generated by transduction with a lentiviral vector coding for mutant and wild-type IDH1[1]. MRS studies were performed on a 500-MHz INOVA spectrometer (Agilent, Santa Clara, CA, USA) using an MR-compatible cell perfusion (bioreactor) system previously described[2]. The perfusion medium in the bioreactor (100 mL) was composed of normal growth medium during HP ^{13}C MRS acquisitions or normal growth medium in which glucose was replaced with 1-^{13}C glucose [5mM] for thermally polarized ^{13}C MRS acquisitions. 1-^{13}C pyruvic acid (PA) and 2-^{13}C PA (Isotech, Sigma Aldrich, St. Louis, MO, USA) containing 15mM of the OX063 trityl radical (Oxford Instruments, Tubney Wood, Abingdon, Oxfordshire, UK) were hyperpolarized using the Hypersense DNP polarizer (Oxford Instruments)[3]. After 1–1.5h, polarized PA was dissolved in 6 mL isotonic 40 mM Tris-based buffer containing 3.0mM EDTA and injected into the perfusion system within 15 s at approximately 37 ºC and to a final concentration of 5mM HP pyruvate. Following the injection of HP pyruvate, single-transient ^{13}C spectra were acquired every 3 s over a period of 300 s using 5º pulses, 40 k data points and a spectral width of 20 kHz. In studies probing the fate of 1-^{13}C glucose, proton-decoupled ^{13}C spectra were acquired in 15 min intervals by using a 60° pulse and 6 s relaxation delay. Spectra were quantified with ACD/Spec Manager version 9.15 software (Advanced Chemistry Development, Toronto, ON, Canada). For HP data, peak integrals were normalized to cell number and to the maximum intensity of the HP pyruvate signal. For thermal polarization data was corrected for saturation and NOE, and normalized to cell number and initial 1- ^{13}C glucose concentration in the culture medium.

Results and discussion: The metabolism of live U87IDHmut and U87IDHwt cells was probed by HP and thermally polarized ^{13}C MRS. HP ^{13}C MR spectra show 2-^{13}C pyruvate conversion to 2-^{13}C lactate (figure 1A) and 5-^{13}C glutamate (figure 1B). U87IDHmut cells displayed a drop by 25% in 5-^{13}C glutamate production (figure 2A). HP ^{13}C MR spectra show 1-^{13}C pyruvate conversion to 1-^{13}C lactate and 1-^{13}C alanine (figure 1C). U87IDHmut cells displayed an increase by 159% ($p<0.001$) in lactate production and by 35% ($p<0.01$) in alanine production (figure 2 C&D). TCA cycle flux was also probed by replacing glucose in the medium with 1-^{13}C glucose and using ^{13}C MRS to monitor the build-up of 4-^{13}C glutamate (34.2 ppm) over a 7-hour period (figure 1D). Glutamate production was significantly down by 64% ($p<0.01$) in mutant IDH cells (figure 2B), whereas glucose uptake was not significantly different (figure 2E). The ^{13}C MRS results are in line with the lower concentration of glutamate (57% $p<0.01$) detected in U87IDHmut cell extracts by ^{1}H NMR spectroscopy. The increase in HP lactate and alanine production and the decrease in HP and thermal glutamate production in IDH1 mutant cells indicate that TCA down-regulation is a major effect of the IDH1 mutation. This down-regulation may be associated with a selective advantage for cancer cells enabling nutrients to be converted to building blocks to be used for proliferation rather than being oxidized in the TCA. Understanding these metabolic fluxes is essential for determining the parameters associated with tumor progression and for the potential development of treatments for mutant IDH-expressing gliomas.

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References: 1.Chaumeil, M. M. *et al. Nat Commun* 4, 2429 (2013). 2.Brandes, A. H. et al. Breast Cancer Research 12, R84 (2010).

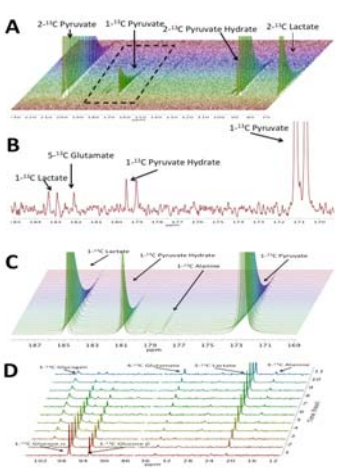

Figure 1: Dynamic HP ^{13}C MR spectral array after 2-^{13}C pyruvate (A) and 1-^{13}C pyruvate (C) shots. Spectra in dotted area (170-185ppm) were added to generate (B). ^{13}C spectral array (D) depicting the build-up of 4-^{13}C glutamate in perfused U87 cells over a 7-hour period.

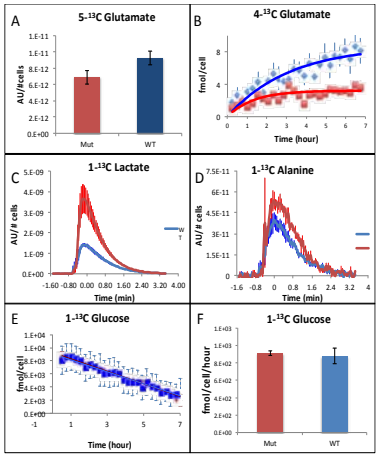

Figure 2: Metabolic differences between U87IDHwt (blue) and U87IDHmut (red) cells: 5-^{13}C glutamate signal after 2-^{13} C pyruvate shot (A); build-up of *de novo* 4-^{13}C-glutamate (B) from 1-^{13}C-glucose uptake (E&F); hyperpolarized lactate (C) and alanine (D) from 1-^{13} C pyruvate.