Reduced production of hyperpolarized 5-13C-glutamate is associated with the IDH1 mutation

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Background: Mutations in isocitrate dehydrogenase 1 (IDH1) have been reported as an early oncogenic event in over 70% of low-grade gliomas and secondary glioblastomas. IDH1 is the enzyme that catalyzes the conversion of isocitrate to -ketoglutarate (-KG) while mutant IDH1 converts -KG to the "oncometabolite" 2-hydroxyglutarate (2-HG). We recently showed that introduction of the IDH1 R132H mutation into two genetically engineered cellular models of varied origins, U87 glioblastoma and immortalized normal human astrocytes (NHA), lead to metabolic reprogramming[1], and a previous study from our laboratory suggested that a decrease in pyruvate dehydrogenase (PDH) activity occurred in NHA IDH1 mutant cells[2]. We therefore set out to determine whether the drop in PDH activity is a general feature of mutant IDH1-associated metabolic reprogramming, and whether it results in a hyperpolarized (HP) ^{13}C MRS-detectable drop in glutamate production in IDH1 mutant cells. Furthermore, we used dichloroacetate (DCA), a PDH agonist, to probe the effect of PDH activation on HP glutamate production and clonogenic potential of our cells.

Methods: The IDH1 wild-type and R132H mutant genes were transduced into U87 and NHA cell lines using lentiviral vectors as described earlier[3]. Sodium dichloroacetate (Sigma Aldrich, MO, USA) was dissolved in water and added to cells at a final concentration of 10 mM 24 h before MRS studies. Clonogenicity was measured using the soft agar assay[4]. MRS studies were performed on a 500-MHz INOVA spectrometer (Agilent, CA, USA) using an MR-compatible cell perfusion (bioreactor) system as previously described[5]. 2-^{13}C pyruvate (Sigma Aldrich, MO, USA) containing 15 mM of the OX063 trityl radical (Oxford Instruments, Oxfordshire, UK) was hyperpolarized using the Hypersense DNP polarizer (Oxford Instruments). After ~1 h, polarized pyruvate was dissolved in 6 mL of isotonic buffer (40 mM Tris-HCl, 3 mM EDTA, pH 7.8) and injected into the perfusion system within 15 s at approximately 37 ºC and to a final concentration of 5 mM 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. Spectra were quantified with ACD/Spec Manager version 9.15 software (Advanced Chemistry Development, ON, Canada). Peak integrals were normalized to cell number and to the maximum intensity of the HP pyruvate signal.

Results and Discussion: HP ^{13}C MR spectra showed metabolism of 2-^{13}C-pyruvate to 5-^{13}C-glutamate (Fig 1A). We observed a significant reduction in 5-^{13}C-glutamate production in U87 and NHA IDH1 mut cells (24±1% [p<0.01] and 76±2% [p<0.001] respectively) relative to their IDH1 wt counterparts (Fig 1B). We also found that, as previously shown for NHA IDH1 mut cells[2], PDH activity is reduced in U87 IDH1 mut cells compared to IDH1 wt cells (Fig 1C). These findings are consistent with observations by us and others that glutamate steady state levels are significantly reduced in IDH1 mutant cells compared to wild-type counterparts[1.] In addition, we confirmed that activation of PDH by DCA treatment caused a significant increase in 5-^{13}C-glutamate production (Fig 2A; 142±30% [p<0.05] for U87 IDH1 mut, 590±146% [p<0.001] for NHA IDH1 mut, 43±5% [p<0.05] for U87 IDH1 wt with a trend to an increase at 22±6% [p=0.14] for NHA IDH1 wt). Furthermore, when we tested the effect of DCA on clonogenicity (Fig 2B), we found that while DCA inhibited clonogenicity of U87 IDH1 wt and mut cells equally (44.6±10.9% for U87 IDH1 wt vs. 49.2±3.6% [p>0.5] for U87 IDH1 mut), this was not the case in the NHA model. NHA IDH1 mut cells were significantly more sensitive to DCA than NHA IDH1 wt cells (85.8±9.6% for NHA IDH1 wt vs. 44.6±3.1% [p<0.05] for NHA IDH1 mut). This probably reflects the greater dependence of NHA IDH1mut cells on the drop in PDH activity for their clonogenic potential, consistent with the fact that the NHA cells are not a fully transformed cell line (in contrast to the U87 cells) and illustrates the role that mutant IDH1-driven metabolic reprogramming plays in tumorigenesis. In summary, our results indicate that PDH down-regulation is likely a significant metabolic consequence of the IDH1 mutation and that HP 5-^{13}C-glutamate can serve as a metabolic imaging biomarker of PDH down-regulation in IDH1 mutant glioma cells.

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References: 1. Izquierdo-Garcia *et al.* ISMRM Milan, 2934 (2014). 2. Izquierdo-Garcia *et al.* PLoSOne 9(9) e108289(2014). 3. Chaumeil *et al. Nat Commun* 4, 2429 (2013). 4. Sonoda *et al.* Cancer Res 61:4956–60 (2001). 5. Brandes *et al*. Breast Cancer Research 12, R84 (2010).

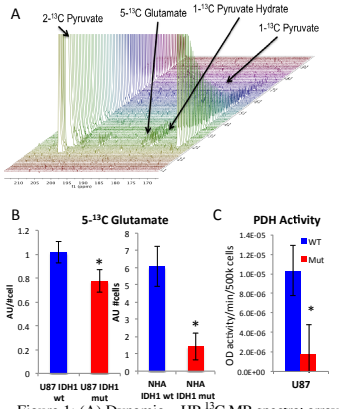

Figure 1: (A) Dynamic HP ^{13}C MR spectral array after 2-^{13}C pyruvate shot. (B) 5-^{13}C glutamate production in IDH1 wt (blue) and IDH1 mut (red) U87 and NHA cells. (C) PDH activity in U87 cells

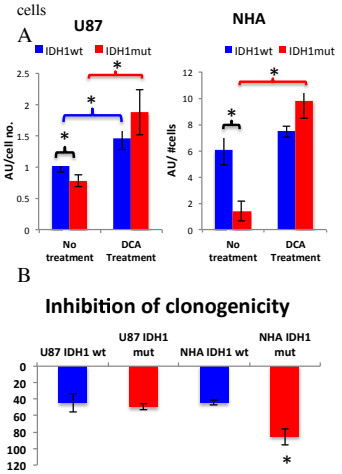

Figure 2: (A) Effect of DCA on 5-^{13}C glutamate production in IDH1 wt (blue) and IDH1 mut (red) U87 and NHA cells. (B) Effect of DCA on clonogenicity of IDH1 wt (blue) and IDH1 mut (red) cells for U87 and