Glutamine is the main source of 2-HG production in IDH1 mutant glioma cells

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Background: 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 in combination ^{13}C-labeled glucose and glutamine to determine the metabolic precursor of 2-HG in mutant IDH1 glioma cells.

Material and Methods: U87 GBM 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 coding for mutant and wild-type IDH1[1]. MRS studies were performed on a 500-MHz INOVA spectrometer (Varian, Santa Clara, CA, USA) using an MR-compatible cell perfusion (bioreactor) system previously described[2]. 1x10^6 cells were seeded on Biosilon microcarrier beads (Nunc, Rochester, NY, USA), allowed to adhere for 24h and loaded into the bioreactor [2]. The perfusion medium in the bioreactor (100 mL) was composed of normal growth medium in which glucose and glutamine were replaced with [1-^{13}C] glucose [5mM] and [3-^{13}C] glutamine [2mM]. Proton-decoupled ^{13}C spectra were acquired in 15 min intervals using a 60° pulse and 6-second relaxation delay. Spectra were quantified with ACD/Spec Manager version 9.15 software (Advanced Chemistry Development, Toronto, ON, Canada). Peak integrals obtained by deconvolution were normalized to cell number and to the initial 1-^{13}C glucose concentration in the culture medium. Data were corrected for saturation effects by using correction factors obtained from a fully relaxed spectrum.

Results: The metabolism of live U87IDHmut and U87IDHwt cells was probed in real time by replacing glucose and glutamine in the medium with their ^{13}-C-labeled counterparts and using^{13}C MRS to monitor the build-up of 2-HG over a 5-hour period. 2-HG produced from glutamine should be labeled on carbon 3 at 32.00 ppm whereas 2-HG produced from glucose should be labeled on carbon 4 at 36.06 ppm. ^{13}C MR spectra of U87IDHmut cells (Fig.1) show the 3-^{13}C 2-HG (32.00 ppm) build-up, generated from 3-^{13}C glutamine, and the absence of 4-^{13}C 2-HG (36.06 ppm) signal. Consistent with this observation, the build-up of glutamate and 2-HG generated from 3-^{13}C glutamine in mutant and wild type cells (Fig. 2A and B respectively) demonstrate that the production of 3-^{13}C 2-HG is significantly higher in mutant cells whereas the build-up of 3-^{13}C glutamate was significantly lower in mutant versus wild-type IDH cells.

Conclusions: This study demonstrates that glucose does not contribute significantly to 2-HG production and that glutamine is the main source of 2-HG in our mutant IDH1 cells, consistent with a previous mass spectrometry study. Furthermore the intracellular glutamate pool is smaller since it is converted first to α kKG and then to 2-HG. Understanding these metabolic fluxes are 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).

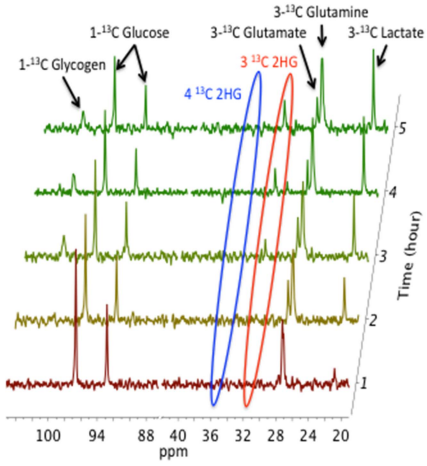

Figure 1:^{13}C spectral array depicting the build-up of 3-^{13}C 2-HG and the absence of 4-^{13}C 2-HG in perfused U87IDHmut cells over a 5-hour period.

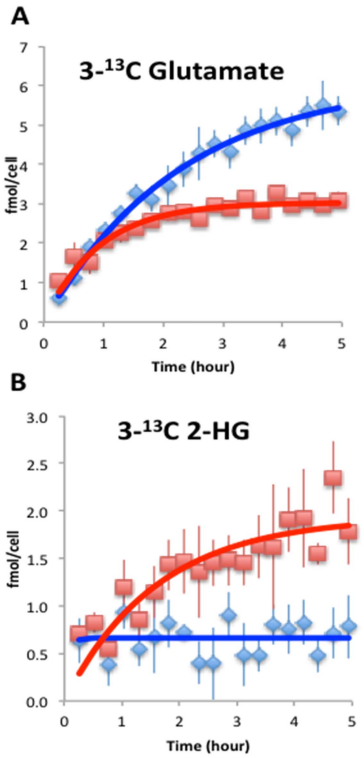

Figure 2: Graph of build-up of *de novo* synthesized 3-^{13}C-glutamate and 2-HG in U87IDHwt (blue) and U87IDHmut (red) cells over 5 hours of exposure to 3-^{13}C-glutamine. The data represent an average of three repeats.