Non-invasive assessment of IDH status in glioblastoma using dynamic ^{13}C MRS of hyperpolarized α-ketoglutarate

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INTRODUCTION

Mutations in the isocitrate dehydrogenase (IDH) enzyme have recently been reported in over 70-80% of low grade gliomas and upgraded glioblastomas (GBM) [1]. Interestingly, the IDH mutation is associated with better prognosis in GBM patients, and thus requires less aggressive therapy. Inhibitors of mutant IDH are under development as novel approaches for glioma treatment. Monitoring IDH status and activity is thus essential for the development and monitoring of personnalized treatment. Wild type IDH catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG), mutant IDH catalyzes the reduction of α-KG into 2-hydroxyglutarate (2-HG) (Fig. 1). To date, IDH status has been assessed using ^{1}H magnetic resonance spectroscopy (MRS) methods aimed at detecting 2-HG accumulation in patients *in vivo* [2] as well as in biopsy samples [3]. However, these methods can be technically challenging and alternative non-invasive techniques are needed. The goal of this study was to evaluate the potential of ^{13}C MRS of hyperpolarized (HP) α-KG as a probe to inform on IDH mutational status in glioma models.

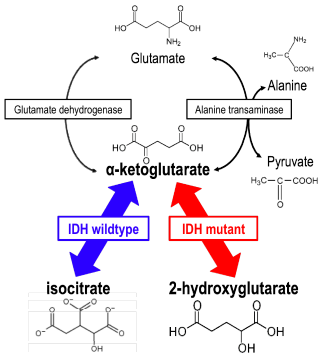

Figure 1 – Metabolism of α-ketoglutarate, showing its conversion into 2-HG in IDHmut cells, and into isocitrate in IDH wt cells

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). Both U87 strains were cultured under standard conditions in supplemented DMEM at 37°C in 5% CO_2. Presence of mutant and wild-type enzyme was confirmed by western blot analysis and by measuring enzyme activity by adapting a previously described spectrophotometric assay [5].

MR of cell lysates 1.3x10^{8} U87IDHmut and U87IDHwt cells were extracted in 1.2mL of lysis buffer as previously described [6,7]. The solution was homogenized and centrifuged; the supernatant was placed in a 10mm NMR tube for further HP studies.

Perfused cell system Cells were grown on microcarrier beads, (3.2±0.3x10^{7} U87IDHwt cells, n=3; 3.1±0.1x10^{7} U87IDHmut cells, n=3), and beads were loaded into a 10-mm NMR tube connected to a perfusion system as previously described [7].

Hyperpolarization of α-KG A volume of 6.4μL (for cell lysates) or 30μL (for perfused cells) of [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. Following polarization, α-KG was rapidly dissolved in Tris -based buffer (pH 7.8) and injected, depending on the experiment, either into cell lysates (to final concentration of 4.7mM) together with 11mM NADPH (NADPH is the co-factor required for IDH activity), or into live perfused cells (to a final concentration of 15mM).

^{13}C MRS acquisition and analysis Following addition of HP α-KG to cell lysates or perfused cells, dynamic sets of HP ^{13}C spectra were acquired on a 500-MHz INOVA spectrometer (Agilent Technologies) with 13 deg excitation pulses and 3 sec TR. After decay of the HP signal, thermal spectra were acquired for an hour (20 degree pulse, 2 sec TR, 400 averages, 5 scans). The resulting spectra were quantified by peak integration using ACD/Spec Manager or AMARES in jMRUI.

RESULTS & DISCUSSION

Western blot analysis confirmed a high level of mutant IDH in the U87IDHmut cell line and a high level of wtIDH in U87IDHwt (Fig. 2A). In line with these results, the rate of mutant IDH activity was >5 times higher in U87IDHmut cells (n=8) vs U87IDHwt (n=7), as shown in Figure 2B (0.610±0.046 fmol/cell/min for U87IDHmut vs 0.100±0.046 fmol/cell/min for U87IDHwt, $p<0.001$).

In U87IDHmut cell lysates, production of HP 2-HG and HP glutamate could be detected at 183.8ppm and 177.2ppm, respectively, following injection of HP α-KG. Of note, an additional peak was also detected at 184 ppm and was identified as a contaminant present in the α-KG preparation. Peak assignments were confirmed by 2D MRS (data not shown). Normalization of spectra to the contaminant shows temporal accumulation of 2-HG in the U87IDHmut lysate (Fig. 3A, note that the increase in noise level is due to the decay of the contaminant hyperpolarization). In contrast, in U87IDHwt lysates, only HP glutamate was visible. Dynamic thermal ^{13}C MR spectra acquired post decay of the HP signal confirm continuous production of 2-HG and glutamate in the U87IDHmut cell lysates, and production of glutamate only in the U87IDHwt cell lysates (Figure 3B). In line with the lysate studies, injection of HP α-KG in live perfused U87IDHwt cells resulted in build-up of HP glutamate (Fig. 4), which reached its maximum 20±8sec after the maximum HP α-KG. In contrast, no HP glutamate could be detected in perfused U87IDHmut cells (n=3) within the time frame of the HP study. Due to the presence of the above-mentioned contaminant (see Fig. 3), HP-2-HG could not be resolved unequivocally in the live cell studies. Studies using alternative HP α-KG sources are underway.

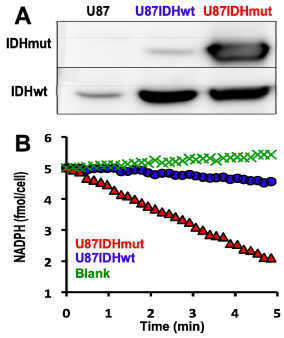

*Figure 2 – **(A)** Western Blotting of IDHmut and IDHwt in U87IDHmut and U87IDHwt GBM cell lines **(B)** Spectrophotometric assay of IDHmut activity over time*

This study demonstrates, to our knowledge for the first time, that HP α-KG permeates the cell membrane, enabling the investigation of its metabolism within a time frame compatible with a ^{13}C MRS experiment. Furthermore, HP α-KG is a promising agent for interrogation of IDH mutational status. This approach could prove useful for personalizing patient care and for development and validation of IDH-targeted therapies.

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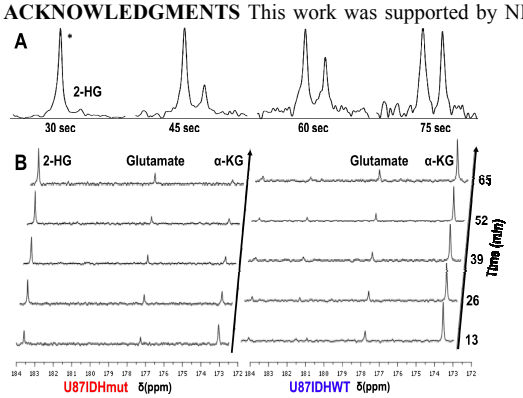

*Figure 3 – **(A)** Evolution of 2-HG in U87IDHmut lysate normalized to contaminant (*) from HP α-KG solution **(B)** Dynamic ^{13}C MRS of cell lysates at thermal equilibrium*

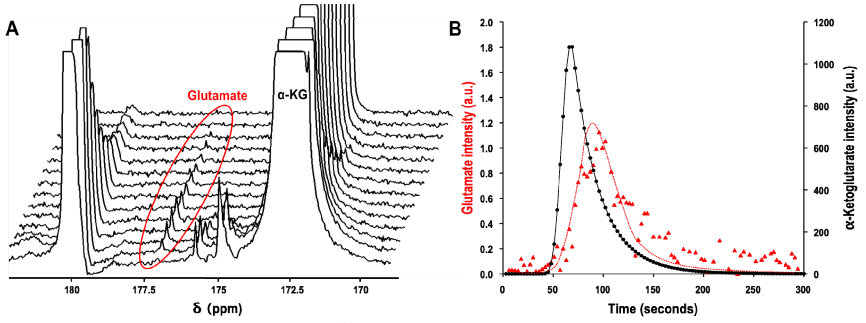

*Figure 4 – **(A)** Stack plot of dynamic HP ^{13}C MR spectra following injection of HP α-KG in U87IDHwt (temporal resolution 9 sec), showing the formation of HP glutamate. **(B)** Corresponding intensities of HP α-KG and HP glutamate, showing the delayed formation of HP glutamate versus HP α-KG.*