MR detectable metabolic changes associated with mutant IDH

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Introduction: Isocitrate dehydrogenase (IDH) is the enzyme within the Kreb's cycle that catalyzes the conversion of α–ketoglutarate (α–KG) into isocitrate. Recently, it was discovered that that the IDH gene is frequently mutated in glioma and leukemia patients. The mutant form of IDH catalyzes the reduction of α–KG into 2-hydroxyglutarate (2HG) and the IDH mutation has been established as an early event in the etiology of glioma (1). Recent high resolution magic angle spinning (HR-MAS) studies of human glioma biopsy samples have shown that the IDH mutation is associated not only with an accumulation of 2HG, but also with several other metabolic changes (2). The goal of this study was to further investigate the MRS-detectable metabolic changes associated with mutant IDH by focusing on two isogenic cell lines that differ only in their IDH status.

Methods: *Cells* U87 human glioblastoma cell lines were virally transduced to produce U87IDHmutant cells carrying the R132H mutant gene and U87IDHwild-type cells carrying an additional copy of the wild-type gene. Both cell lines were cultured in supplemented high glucose DMEM at 37°C in 5% CO_2.
Cell extract studies Cell extraction was performed using the dual phrase extraction method. In brief, cells were trypsinized and vortexed in the presence of ice-cold methanol followed by chloroform and water. Cell lysates were then spun down and the methanol/water phase lyophilized, dissolved in D_2O, and ^1H MRS performed using a 600MHz Varian (Agilent) INOVA spectrometer using a 90° flip angle and a 3 second relaxation delay. Concentrations of metabolites were determined by integration, correction for saturation, and normalization to cell number and an external TMSP reference of known concentration.
Activity Assay and Western Blot Analysis Expression levels of monocarboxylate transporters 1 and 4 (MCT 1, MCT 4) were assessed by Western Blot analysis. Enzymatic activity of lactate dehydrogenase A (LDHA) was measured as previously described (3). Briefly, an Infinite M200 spectrophotometer was used to monitor the consumption of NADH in the presence of different concentrations of pyruvate. Lineweaver-Burke plot analysis was used to determine values of V_{max}.
Live cells studies To monitor cell metabolism, cells were grown on Bioslin beads and loaded into an NMR-compatible perfusion system as previously described (3). Briefly, three tubing lines circulated medium to a 10mm NMR tube and an additional tube supplied 5% CO_2. A three-way valve allowed for the introduction of hyperpolarized substrate. Samples of [1-^{13}C] pyruvic acid were hyperpolarized using the Hypersense DNP polarizer. After approximately 1 hour, pyruvic acid was dissolved in Tris-based buffer and injected into the perfusion system over 20 seconds. ^{13}C spectra were acquired every 3 seconds for 300 seconds using 13° excitation pulses. The intensities of lactate and pyruvate peaks were determined using ACD software and normalized to total hyperpolarized signal and cell number. Lactate-to-pyruvate ratio (Lactate/Pyruvate) was calculated as the ratio of maximum lactate signal to maximum pyruvate signal (3).

Results: ^{1}H MRS of cell extracts confirmed the presence of 2HG in mutant but not in wild type IDH cells (Fig. 1). In addition, several other metabolic changes were observed (Fig. 2). Most notably, significant increases in phosphocholine (PC) and total choline containing metabolites (tCho) were observed, consistent with the elevation of tCho reported in patient samples (2). An elevation in intracellular lactate levels was also observed in mutant IDH cells. To further investigate the elevation in intracellular lactate we probed the pyruvate to lactate conversion in live cells cultured in a bioreactor system. Unexpectedly, pyruvate to lactate conversion was lower in mutant IDH cells when compared to control (Fig. 3a). The lactate-to-pyruvate ratio dropped to 31 % from 3.7±0.9 in wild type IDH cells to 1.2±0.3 in mutant IDH cells ($p < 0.02$ Fig. 3b). The pyruvate to lactate conversion can be modulated by several factors including the activity of LDH, which catalyzes the pyruvate to lactate conversion, and the expression of MCT 1 and MCT 4 transporters, which control transport of pyruvate and lactate in and out of the cell. Preliminary studies indicate that LDH activity, as assessed by cellular $V_{max,}$ drops in mutant cells to 33% of wild type from 12.9±4.8 µmoles NADH/min/10^7 cells to 4.3±1.9 µmoles NADH/min/10^7 cells (n=3, $p=0.03$, Figure 4a.). This result provides a likely mechanism for the drop seen in the hyperpolarized pyruvate to lactate conversion and indicates that the elevation in MCT1 expression (Figure 4b.) is likely not playing a rate-limiting role in our cells. The drop in MCT4 could provide an explanation for the elevated level of intracellular lactate observed in IDH mutant cells (Figure 4b.).

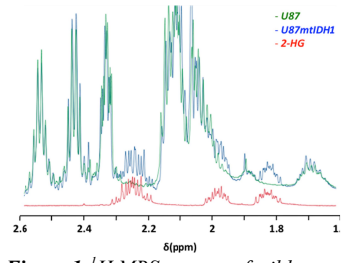

Figure 1. *^{1}H MRS spectra of wild-type (green) and IDH mutant (blue) U87 cells. The spectrum in red is from pure 2-HG.*

Discussion and Conclusions: The mechanism through which mutant IDH mediates oncogenesis remains to be fully elucidated. Recent studies indicate that 2HG is a competitive inhibitor of several α-KG-dependent dioxygenases (including histone demethylases and the TET family of 5-methylcytosine hydroxylases). Elevated 2HG can therefore lead to changes in genome-wide histone and DNA methylation that can contribute to oncogenesis via multiple pathways. Our studies indicate that metabolic enzymes are included amongst the genes altered by mutant IDH expression resulting in a range of MRS detectable metabolic changes beyond the expected presence of 2HG. These metabolic changes inform on the wide-ranging metabolic consequences of the IDH gene mutation and could, collectively, serve as biomarkers of the presence of mutant IDH.

References. 1. Gupta et al J Clin Pathol 2011;64:835. 2. Elkhaled A et al. ISMRM; 2011. p. 182. **3.** Ward et al Cancer Res. 2010;70:1296-305.
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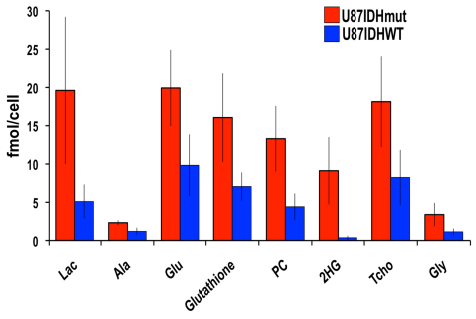

Figure 2. *Summary of significant metabolic changes detected in the ^{1}H MRS spectra of U87IDH wild-type (blue n=5) and U87 IDH mutant (red, n=5) cell extracts.*

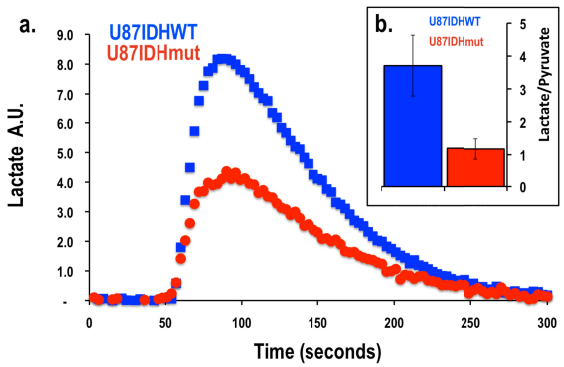

Figure 3a. *Hyperpolarized lactate production in U87 IDH wild type (blue) and U87 IDH mutants (red) perfused cells.* ***3b.*** *Average lactate-to-pyruvate ratios in U87 IDH wild type (blue, n=3) and U87 IDH mutants (red, n=3) perfused cells.*

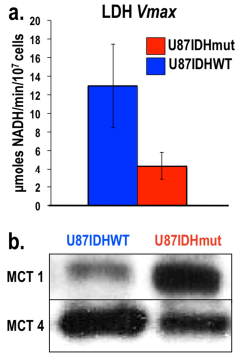

Figure 4a. *Results of LDH Activity assay* ***4b.*** *Western blot analysis showing the effects of IDH mutation on MCT1 and MCT4 expression.*