The tumor exception that proves the rule: Hyperpolarized ^{13}C MRS cannot be used to detect the presence of mutant IDH1 glioma or their responses to Temozolomide therapy

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INTRODUCTION

The most prevalent mutation in brain tumors (found in >70% of glioma and 90% of secondary glioblastoma (GBM)), affects isocitrate dehydrogenase 1 (IDH1) [1,2]. Importantly, the IDH1 mutation is associated with epigenetically-driven modulations of metabolism[2]. In particular, we recently demonstrated that the lactate dehydrogenase A (LDH-A) enzyme was silenced in mutant IDH1 gliomas, highlighting the unusual metabolism of these tumors [3].

Temozolomide (TMZ), the standard of care chemotherapy for primary GBM patients [4,5], is currently being investigated as a therapeutic agent for Grade II and III gliomas with promising results [5,6]. Recent studies of primary (wild-type IDH1) GBM cells and tumors demonstrated that ^{13}C MRSI of hyperpolarized (HP) [1-^{13}C] pyruvate could be used as an early indicator of TMZ response [7-8]. However, to date, this metabolic imaging method has never been applied to the study of mutant IDH1 Grade II or III tumors. Given their uncharacteristic metabolism, and based on the importance of monitoring TMZ response in these tumor types, we decided to investigate the relevance of ^{13}C MRSI of HP [1-^{13}C] pyruvate in Grade III gliomas. Two recently developed models of Grade III glioma, one oligoastrocytoma (OA) and one oligodendroglioma (OD) in which LDH-A is silenced, were investigated [3-9], and their response to TMZ therapy evaluated using this neuroimaging method.

MATERIAL & METHODS

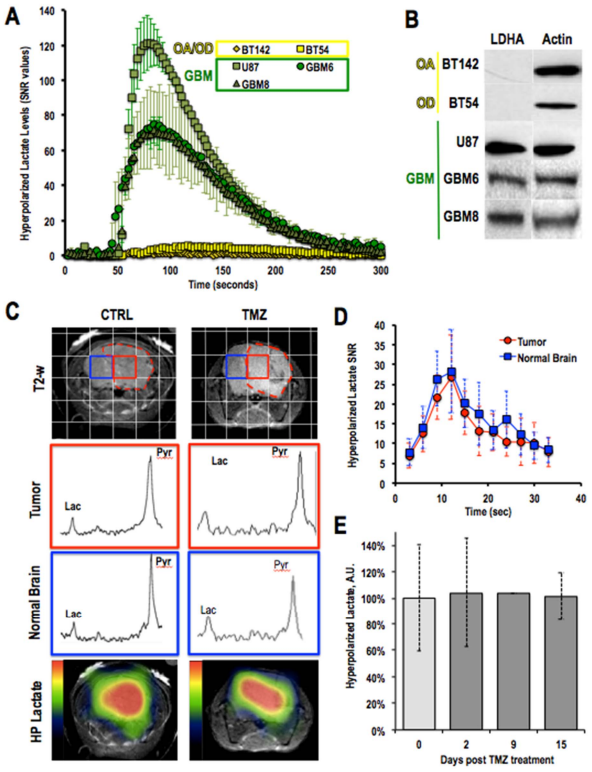
Cells and perfusion system BT142 and BT54 (Grade III OA & AD, resp.) were cultured as neurospheres [3], GBM6, GBM8 and U87 primary GBM cells as monolayers [7,10]. For bioreactors, cells (~3x10^7 cells per study) were encapsulated in agarose beads and loaded in a 10mm tube connected to a perfusion system [10-12].

Tumor-bearing animals BT142 orthotopic tumors were implanted in athymic mice (n=9; n=6 control, n=2 TMZ) by injecting a suspension of cells (~3x10^5 in 3µL) in the right caudate [11-13]. Animals were imaged 90-120 days post-intracranial injection. A 27G catheter was secured in the tail vein for injection of HP agent.

TMZ treatment Once the tumor reached a size of 16mm^3, animals were treated once daily by oral gavage (dose 5mg/kg, v=150µL).

Hyperpolarized [1-^{13}C] pyruvate 6µL (for cells) or 24µL (for animals) of [1-^{13}C] pyruvic acid (Isotec), 15mM OX63 trityl radical (Oxford Instruments, Abingdon, UK) and Dotarem (Gd-DOTA, Guerbet, France) were hyperpolarized using a HyperSense\ DNP polarizer (Oxford Instruments) for approx. one hour [10-12].

^{13}C MRS acquisition and analysis Perfused cells: Dynamic sets of HP ^{13}C spectra were acquired on an 11.7Tesla INOVA spectrometer (Agilent Technologies) using 13deg excitation pulses and 3sec TR. Cell ^{13}C spectra were quantified using ACD/Spec Manager and lactate relative SNR determined by normalizing lactate signal to cell number, maximum pyruvate levels and standard deviation of the noise. *In vivo:* 5sec after the start of iv injection, 2D dynamic CSI ^{13}C data were acquired on a 14.1Tesla MR system (Agilent Technologies) equipped with a ^{1}H-^{13}C coil (Ø$_I$=40mm) using the following parameters: TE/TR=1.2/60ms; SW 2500Hz; 128 points; 4 sec resolution; FA 10deg; FOV 24x24; 5mm slice thickness. *In vivo* data were processed using the in-house SIVIC software [10-12].



***(A)**- HP lactate levels (A.U.) in primary GBM cell lines (n=3 U87, n=3 GBM6, n=2 GBM8, green) and Grade III OA glioma cell lines (n=4 BT142, n=2 BT54, yellow). **(B)** LDH-A protein expression in all cell lines as assessed by western blots **(C)** T2-weighted axial image, HP ^{13}C spectra from a tumor voxel (red) and from a normal brain voxel (blue) and HP lactate heatmap of a control (left) and a TMZ treated (right) BT142 tumor-bearing animal. **(D)** HP lactate kinetics in tumor (red) and normal brain (blue) for of control and TMZ treated BT142 tumor bearing animals (n=4 per group). **(E)** HP lactate levels (% of day 0) as measured in tumors during TMZ treatment (Days 2,9,15).*

RESULTS

In contrast to GBM, mutant IDH1 OA & OD perfused cells do not produce a significant amount of HP [1-^{13}C] lactate from HP [1-^{13}C] pyruvate: Injection of HP [1-^{13}C] pyruvate in the medium of perfused primary GBM cells (n=3 U87; n=3 GBM6; n=2 GBM8) resulted in significant production of HP [1-^{13}C] lactate, as previously reported [6,8,12] (SNR_{U87}=122±14; SNR_{GBM6}=77±21; SNR_{GBM8}=72±6). In contrast, following injection of HP [1-^{13}C] pyruvate in the medium of perfused OA and OD cells (n=3 BT142; n=2 BT54), HP [1-^{13}C] lactate was barely above noise level (SNR_{BT142}=3.5±1.0; SNR_{BT54}=5.7±0.2) and significantly lower than in GBM cells ($p<0.01$). These results were in line with the mutant IDH1-mediated silencing of LDH-A enzyme in OA and OD tumors (Fig. B & ref. 3).

Mutant IDH1 OD gliomas are not detectable *in vivo* following injection of HP [1-^{13}C] pyruvate: Given our cell results, we questioned the relevance of ^{13}C MRSI of HP [1-^{13}C] pyruvate for *in vivo* detection of OD gliomas. As opposed to GBM, HP [1-^{13}C] lactate levels were not significantly different between tumor and normal brain (SNR_{Tumor}=29±9; $SNR_{Normal\ Brain}$=29±5; $p=0.94$; Fig. C&D), making this tumor virtually "invisible" by HP ^{13}C MRSI.

TMZ treatment does not affect HP [1-^{13}C] lactate levels in OA tumors: Finally, we wanted to investigate if, as previously shown in primary GBM tumors [7-8], HP ^{13}C MRSI could be used for early detection of TMZ response in OA tumors. As shown in Fig. C &D, no significant differences in HP [1-^{13}C] lactate levels could be detected between control and TMZ-treated animals at any time point post TMZ treatment ($p>0.8$ for all time points) even though TMZ treatment appeared to lead to an increase in survival and tumor shrinkage (n=2).

DISCUSSION & CONCLUSIONS

Mostly due to the lack of available models, every preclinical study of brain tumors using HP [1-^{13}C] pyruvate had, to date, been performed on primary GBM tumors [7, 11-13]. To our knowledge, this is the first report of the use of ^{13}C MRSI of HP [1-^{13}C] pyruvate in mutant IDH1 OA or OD glioma. We demonstrate that, in contrast to GBM tumors that produce high levels of HP [1-^{13}C] lactate, mutant IDH1 OA and OD gliomas are virtually "invisible" post injection of HP [1-^{13}C] pyruvate, in perfused cells and *in vivo*. Our findings are consistent with the relatively low level of lactate observed in OA and OD tumors by ^{1}H MRS (and also observed in BT142 tumors, data not shown). Furthermore, our findings highlight the unusual metabolism of mutant IDH1-driven OA and OD tumors.

Acknowledgements: NIH R21CA16154, NIH R01CA172845, NIH P41EB013598, Alberta Innovates Health Solutions and Alberta Cancer Foundation **References**: 1. Yang, Clin Cancer Res (2012); 2. Huse, Glia (2011); 3. Cheneslong, NeuroOnc (2014); 4. Osoba D, J Clin Oncol (2000); 5. Johnson DR, J Neurooncol (2011); 6. Friedman HS, Clin Cancer Res (2000); 7. Park & Mukherjee, Cancer Res (2014); 8. Park et al, JMRI (2011); 9. Luchman, NeuroOnc (2012) 10. Ward, Cancer Res (2010); 11. Chaumeil, Nature Comm (2013); 12. Chaumeil, Cancer Res (2014); 13. Chaumeil, Neuroimage (2012).