Hyperpolarized 13C MR Metabolic Imaging Provides an Early Biomarker of MGMT Activity and Response to Temozolomide Treatment

I. Park[1], L. E. Jalbert[1], T. Ozawa[2], C. James[2], J. J. Phillips[2], D. B. Vigneron[1,3], R. O. Pieper[2], S. M. Ronen[1], and S. J. Nelson[1,3]

[1]Surbeck Laboratory of Advanced Imaging, Department of Radiology and Biomedical Imaging, University of California, San Francisco, San Francisco, CA, United States, [2]Brain Tumor Research Center, Department of Neurological Surgery, University of California, San Francisco, San Francisco, CA, United States, [3]Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, CA, United States

Introduction: Although a number of studies have indicated that high levels of DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT) predict response to Temozolomide (TMZ) therapy in gliomas [1], there is no non-invasive method for measuring MGMT activity in tumors *in vivo*. A recent study has shown that tumor metabolism can be examined in an orthotopic human xenograft model using DNP-hyperpolarized ^{13}C MRSI [2]. The purpose of this study was to demonstrate that early TMZ-induced DNA damage modulates pyruvate metabolism in the absence, but not in the presence, of MGMT through a mechanism independent of apoptosis, and therefore to demonstrate that hyperpolarized ^{13}C MR metabolic imaging using [1-^{13}C]-pyruvate can provide an early biomarker of MGMT activity and response to TMZ treatment.

Methods: In order to assess the effect of MGMT on pyruvate metabolism in an orthotopic human glioblastoma xenograft model, MGMT-proficient U87-MG cells were produced from MGMT-deficient U87-MG cells through lentiviral infection encoding MGMT and subsequent sorting by flow cytometry (Figure 1) [3]. Twenty-three athymic rats with intracranial implantation of either MGMT-deficient U87-MG (MGMT−) or MGMT-proficient U87-MG (MGMT+) cells were divided into four groups: MGMT− treated group (n=10) and MGMT+ treated group (n=4) received an oral administration of 100 mg/kg TMZ, and MGMT− control group (n=10) and MGMT+ control group (n=3) received the vehicle only. All animals underwent ^{13}C and ^{1}H imaging study prior to treatment (D0), at D1 (days from treatment) and/or D2. All imaging studies were performed using a GE 3T scanner with a custom-designed $^{1}H/^{13}C$ rat coil. ^{13}C 3D MRSI data (TE/TR=140/215 ms, 4x4x5.4 mm resolution) were acquired using a double spin echo sequence with centric k-space encoding, a variable flip angle scheme and echo-planar readout [4] at 20 sec after the injection of approximately 2.5 ml (100 mM) hyperpolarized [1-^{13}C]-pyruvate through the tail vein. T1-weighted axial spin-echo images were obtained following injection of 0.2 mmol/kg Gadolinium (Gd)-DTPA in order to estimate tumor volume. The ratio of lactate over pyruvate (Lac/Pyr) was calculated from the voxel centered at tumor in the ^{13}C spectra for the assessment of change in tumor metabolism. In order to determine whether early response measured by hyperpolarized ^{13}C data was caused by TMZ-induced apoptosis, NAD+/NADH levels and LDH activity were assessed from the resected brains of 8 MGMT− treated and 7 MGMT− control rats after their experiment at D2 using the previously described method [5]. In addition, the degree of apoptosis was estimated by counting the number of tumor cells positive for cleaved caspase-3 staining (per 200x field) for five independent regions.

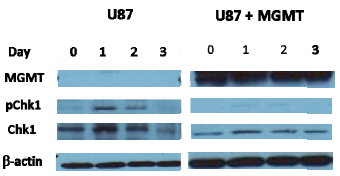

Figure 1. Western blots of treated U87 and U87+MGMT cells confirmed MGMT expression and demonstrated that MGMT prevents TMZ-induced Chk1 activation.

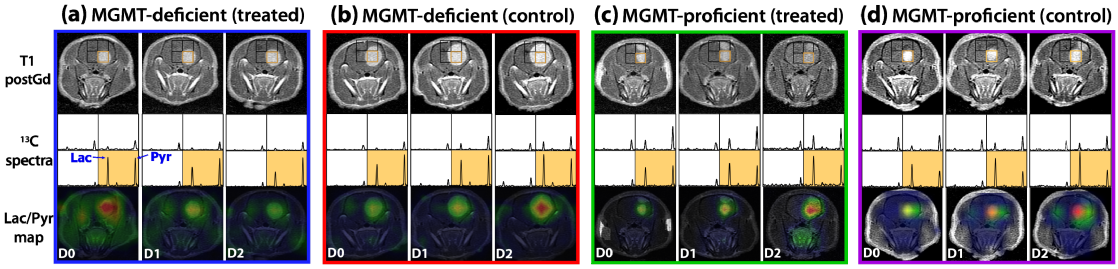

Figure 2. Lac/Pyr dropped with TMZ-treatment at D1 and D2 compared to pre-treatment (D0) in MGMT-deficient treated tumors (a). Lac/Pyr increased in all the other groups.

Results: TMZ treatment in MGMT− tumors caused a rapid and significant drop in Lac/Pyr (Figure 2a), while Lac/Pyr increased in MGMT− control tumors (Figure 2b). In contrast, both treated and control groups with MGMT+ tumors exhibited an increased Lac/Pyr level with findings comparable to MGMT− control tumors (Figure 2c and 2d). On D1, Lac/Pyr dropped by 21±22 % in MGMT− treated rats, but increased 20±25, 16±11 and 23±18 % in MGMT− control ($p<0.004$), MGMT+ treated ($p<0.03$) and MGMT+ control rats ($p<0.02$), respectively. Results from D2 showed similar findings (Figure 3a). In contrast, tumor volume increased over time for all groups ($p>0.2$) (Figure 3b). Tumor shrinkage was observed in MGMT− treated group at later time points between D5 and D7 [6]. The results from biological assays and immunohistochemical analysis indicated that NAD+/NADH levels, LDH activity and the extent of apoptosis were not statistically different between the TMZ-treated and control tumors at day 2 following the initiation of treatment (Figure 4). These findings suggest that the early inhibition of hyperpolarized ^{13}C pyruvate metabolism is specific to O^6-methylguanine lesion caused by TMZ in the absence of MGMT and occurs long before the delayed cell death typical of GBM treated with TMZ.

Conclusions: We have demonstrated that pyruvate metabolism monitored by hyperpolarized ^{13}C MRSI can provide a very fast and reliable way to determine the status of MGMT and predict tumor response to TMZ treatment. The reduction of Lac/Pyr in MGMT-deficient tumors as early as one day after TMZ treatment implies that the altered pyruvate metabolism is independent of the typical delayed cell death induced by TMZ and specific to DNA damage caused in the absence of MGMT. The results from this study suggest that this technique may allow neuro-oncologists to quickly evaluate patient response to TMZ and enable them to tailor customized therapy for individual patients with brain tumors.

References: [1] Hegi et al., *Clin Cancer Res*, 2004;15;10:1871-4 [2] Park et al., *Neuro-Oncol*, 2010;12:133-44 [3] Zielske et al., *Clin Invest*, 2003;112:1561-70 [4] Cunningham et al., *J Magn Reson*, 2007;187:357-362 [5] Dafni H et al., *Cancer Res*, 2010;70:7400-10 [6] Park et al., *Proc. ISMRM, 18th Annual Meeting,* 2010. p 3272

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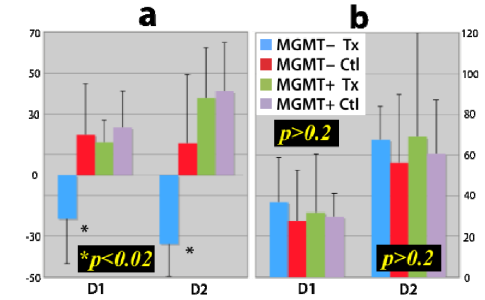

Figure 3. Percent Lac/Pyr (a) and tumor volume (b) change from baseline at D1 and D2 for all groups. MGMT-deficient treated rats (MGMT− Tx) showed a reduction in Lac/Pyr at D1 and D2, while tumor volume increased for all groups.

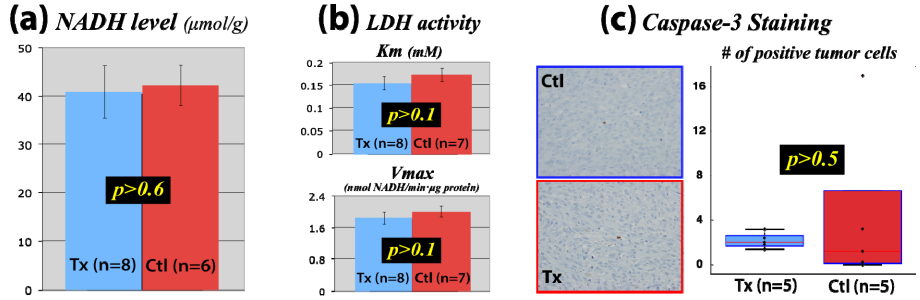

Figure 4. Treated (Tx) and control (Ctl) groups with MGMT-deficient tumors were euthanized at D2 and their brain analyzed for NADH (a), LDH activity assay (b) and caspase-3 staining (b). None of these parameters were significantly different between the two groups at D2, suggesting that the early inhibition of ^{13}C metabolism in MGMT-deficient group is not associated with apoptosis.