Chemotherapy resistant, dormant Glioblastoma cells exhibit high rates of oxidative metabolism

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Introduction: Metabolic imaging (^{18}F-fluoro-deoxyglucose, FDG-PET) is widely used to monitor treatment response of many cancers. However, its use in neuro-oncology has been limited. Although ^{18}FDG-PET imaging can accurately monitor the transition of indolent, low-grade gliomas, to high-grade (Glioblastoma, GBM) disease which is associated with rapid proliferation, it has consistently failed to reflect treatment response when patients are clearly 'stable' from a clinical and radiographic perspective, following concurrent radiation (RT) and chemotherapy (temozolomide,TMZ). It is hypothesized that the transition from indolent to rapid growth, which requires increased metabolic resources to support macromolecule synthesis, is associated with a switch from oxidative metabolism to aerobic glycolysis (Warburg effect). If this is correct, then it is reasonable to hypothesize that the ^{18}FDG-PET metabolic profile post-treatment, stable GBM should reflect a lower bioenergetic demand, similar to a low-grade tumor. This, however, does not reflect clinical experience. Rather, stable GBMs most often show persistent high uptake of ^{18}FDG and this has limited utility of ^{18}FDG-PET in the management of gliomas. Thus there is a discrepancy between non-proliferating GBM and high uptake of ^{18}FDG. Here we directly tested the assumption that fast growing GBM will have a fundamentally different metabolic profile compared to proliferation-arrested tumors. Using a human orthotopic mouse model of GBM (HOT) that shows a cytostatic response to TMZ, we compared the ^{13}C-NMR profiles of TMZ treated and untreated GBM following infusion of ^{13}C-glucose.

Methods: The GBM HOT mouse model was produced by isolating tumor cells from freshly resected human surgical specimens and stereotactically injecting them into 6-week-old NOD/SCID mouse brain. Seven individual HOT lines were used for these studies, 1 mouse for treatment and vehicle from each line. Neurologically symptomatic mice were treated with TMZ at clinically equivalent dose (20 mg/kg x 5 days, equivalent to one cycle of standard care), or vehicle. Tracer studies were performed 3 days later. After a priming bolus, awake mice were infused with [U-^{13}C] glucose for 150 minutes, then deeply anesthetized and cardiac perfused with ice-cold PBS. Tumor and non-tumor bearing brain regions were rapidly dissected and freeze-clamped. Proton–decoupled ^{13}C NMR spectra of tissue extracts were acquired at 150 MHz (600 MHz) with ^{2}H field-frequency lock.

Results: Histopathological analysis confirmed that TMZ induced a dramatic inhibition of GBM cell proliferation in the treated tumors (MiB-1 6% vs vehicle 65%). Representative ^{13}C NMR spectra of tissue extracts are shown in Fig.1. Multiplets in glutamate C4 indicate oxidation of glucose in the citric acid cycle. No significant difference was observed between TMZ treated and untreated (-TMZ) for any line (data not shown). Similarly, the full ^{13}C spectrum including enrichment in lactate (LAC), alanine (ALA), glutamate (GLU) and glutamine (GLN) were similar (data not shown). It is important to note that the absence of significant changes in the metabolic profile of GBM cells in vivo by TMZ induced cell-cycle arrest was associated with clear evidence of DNA damage mediated p53-p21 activation. This observation is remarkable for the fact that p53 is regarded as how tumor cells utilize respiratory and glycolytic pathways.

Conclusion: Deregulation of tumor metabolism has been intimately linked to cell proliferation. Here we report, contrary to expectation, that following chemotherapy induced arrest, dormant GBM continue to exhibit high rates of glucose oxidation in vivo. This observation may in part explain why PET studies have failed to be prognostic of therapeutic response and raises the possibility that metabolic profiles of tumor are determined by their mutational and differentiation status and not simply proliferation rate.

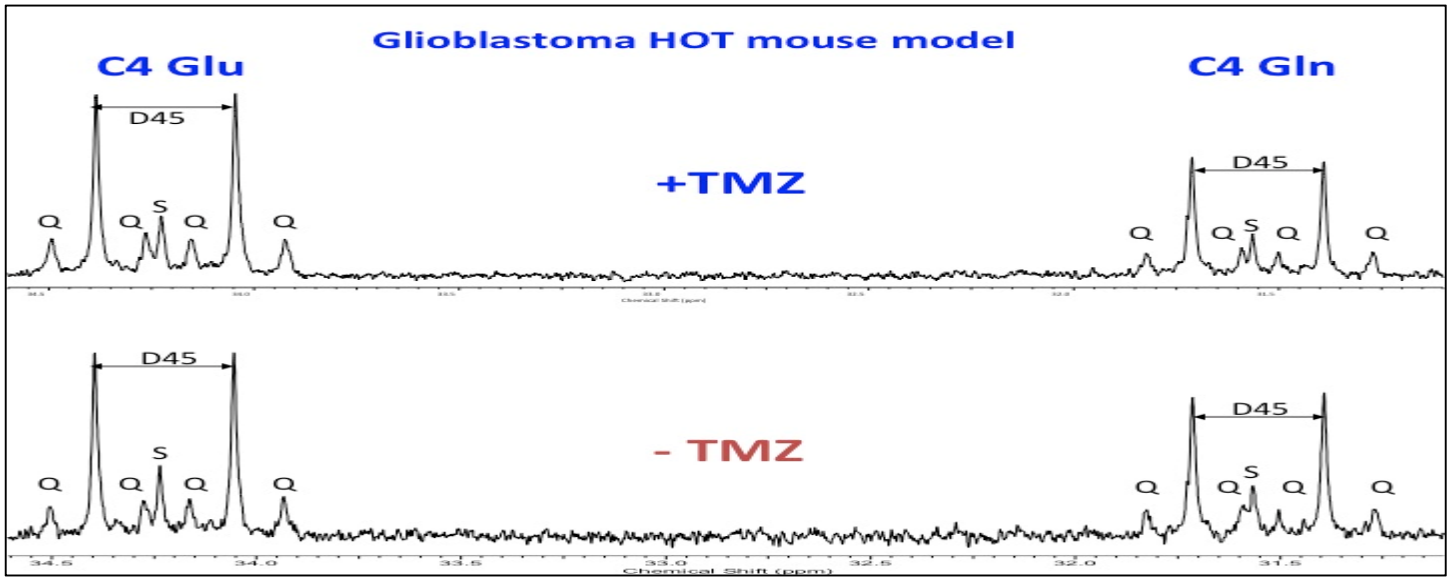

Fig.1 ^{13}C-NMR spectra from TMZ-treated (upper panel) and untreated (vehicle; lower panel). Carbon 4 (C4) of Glutamate (Glu) and Glutamine (Gln) . Similar pattern of multiplets are seen in both spectra. Abbrev: D45 (doublet 45), S (singlet), Q (quartet).