

Beta Oxidation of Octanoate by Diverse Human Primary and Metastatic Brain Malignancies in an Orthotopic Transplant Model

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Target audience: Researchers and clinicians studying metabolism in brain tumors.

Purpose: Brain tumors *in situ* are exposed to intermediate- and long-chain fatty acids, as well as lactate, carbohydrates and other substrates for energy production in mitochondria. The overwhelming majority of studies in tumor metabolism focus on conversion of glucose to lactate, and the preferred model is isolated cells in culture. However, there is growing evidence that cells lines conditioned to culture may not correctly reflect metabolism *in situ*, and some tumors appear capable of oxidizing substrates other than glucose. Furthermore, the enzymes for β -oxidation of fatty acids are present in some malignancies, and there is general agreement that tumor oncogenes reprogram intermediary metabolism. In this study, our previously described human orthotopic tumor (HOT) glioblastoma GBM mouse models were studied by ^{13}C NMR isotopomer analysis to assess whether β -oxidation is active. Since animals (and patients) with advanced malignancies are often undernourished, the effects of hepatic gluconeogenesis on ^{13}C -labeling in plasma glucose, also available to the tumor, was evaluated.

Materials and Methods: All studies were performed with approval of the local Institutional Review Board and Animal Care Committee. Six individual HOT models, all isocitrate dehydrogenase wild type, were used. Expression analysis for the common driver GBM mutations, c-Met, EGFR, P53 and PDGFR α , was performed for each human and paired HOT mouse line and at least one example of each of the common driver mutations was chosen. Three metastatic cancers to the brain were also studied; breast cancer (estrogen receptor and progesterone receptor negative, HER2-neu positive), adenocarcinoma of the lung (EGFR, Ras, BRAF wild type), and endometrial cancer. One mouse from each of the eight representative tumors was examined. MRI was used to monitor tumor growth. When animals had difficulty walking or began to lose weight, MRI and ^{18}F FDG PET were performed, documented the tumor, and the tracer study was performed. Under general anesthesia [^{13}C] octanoate (220mM, 2.5 $\mu\text{L}/\text{min}$) was infused intravenously for 150 min. Liver and brain were rapidly dissected. The liver was freeze-clamped and the brain was placed in iced saline for dissection of the tumor. Tumor and surrounding (non-tumor bearing) brain were freeze-clamped separately. All tissue was extracted with perchloric acid and the soluble fraction from liver, tumor and non-tumor brain from each animal was studied by high resolution ^{13}C and ^1H NMR spectroscopy performed on a 14.1 T Agilent NMR Spectrometer. Histology showed that > 95% of the cells in the tumor mass were malignant.

Results and Discussion: The ^{13}C NMR spectrum of liver glucose was dominated by singlets, although small multiplets, ~3-5% of the total area of each glucose resonance, could occasionally be detected. The lactate methyl resonance from tumor and surrounding brain was < 5% ^{13}C (from the ^1H spectrum) and of this, ~20% was [2,3- ^{13}C] lactate (from ^{13}C spectrum). Therefore, <1% of the pyruvate pool in the brain was [2,3- ^{13}C] pyruvate derived from ^{13}C -labeled glucose from hepatic gluconeogenesis. Spin-coupled multiplets due to ^{13}C - ^{13}C coupling were observed in glutamine or glutamate in all tumors and in all non-tumor bearing brain; examples are shown in the Figure. The C4 resonance of glutamine was dominated by [4,5- ^{13}C] glutamine and about 13% of the signal in C4 was derived from [3,4,5- ^{13}C] glutamine. From Histological data, it was assumed that the tumor was metabolically homogeneous. Isotopomer analysis showed that ~25% of acetyl-CoA was derived from infused octanoate; the remainder was derived from unlabeled substrates. Surprisingly, all tumors, regardless of origin, had preserved capacity to oxidize octanoate.

Conclusions: In mice with orthotopic transplants of diverse human brain malignancies, intravenously-infused [^{13}C] octanoate is oxidized in to acetyl-CoA in both surrounding brain and tumor.

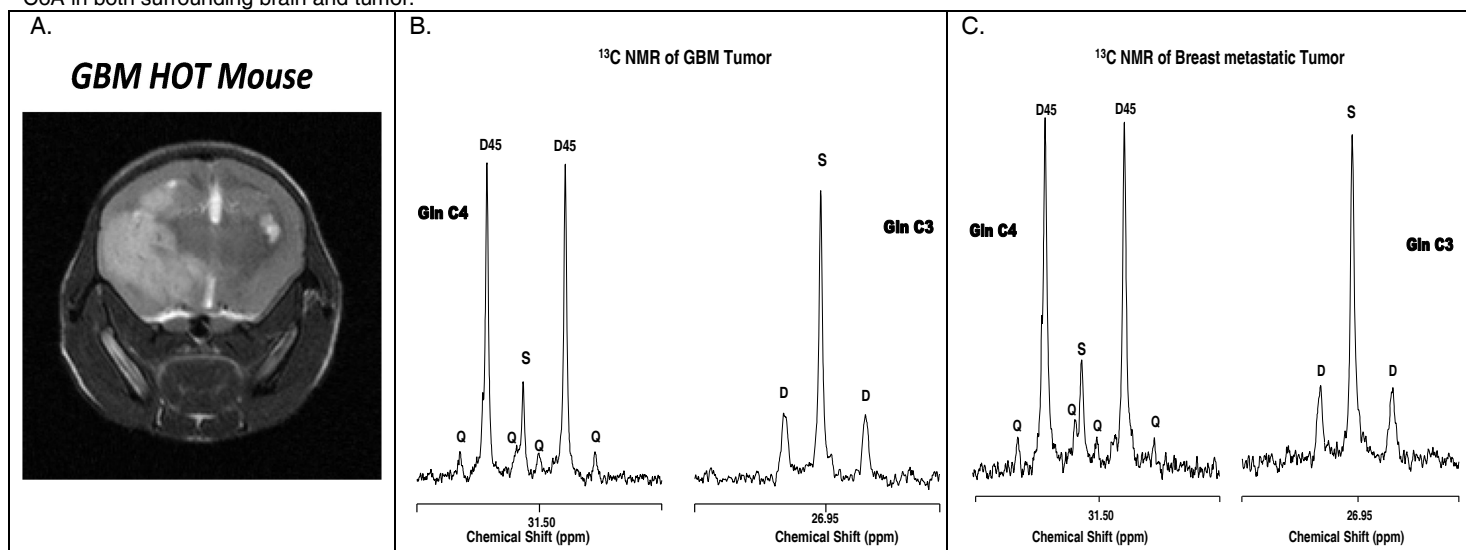


Figure. A) MRI of the mouse brain with implanted GBM. B) High resolution ^1H -decoupled ^{13}C NMR spectrum of glutamine from a GBM. C) High resolution ^1H -decoupled ^{13}C NMR spectrum of glutamine from a metastatic lung cancer. In all instances metabolic products due to oxidation of [^{13}C]octanoate to [1,2- ^{13}C]acetyl-CoA were easily detected. Abbreviations: S, singlet; D, doublet due to the ^{13}C - ^{13}C coupling; Q, doublet of doublets due to ^{13}C - ^{13}C coupling.