Does the Warburg effect exist in vivo? Analyzing glucose metabolism in FDG-PET-positive tumors by 13C-NMR spectroscopy

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Introduction: Tumor cells consume glucose to support bioenergetics and biosynthesis. Enhanced tumor glucose uptake can be imaged by positron emission tomography with 2-deoxy-2-18F-fluoro-D-glucose (FDG-PET), but the final fate of glucose carbon is unknown. It is assumed that anaerobic glucose metabolism is active in tumors, while oxidative pathways in the mitochondria are suppressed (the Warburg effect). On the other hand, glutamine is thought to be the main substrate to replenish citric acid cycle (CAC) intermediates in tumor cells *in vitro*, a process termed anaplerosis. Here we used an orthotopic glioblastoma (GBM) model to probe glucose and glutamine metabolism *in vivo*.

Methods: Human GBM cells were obtained at surgical resection and implanted into the brains of immunocompromised mice. Multiple mice bearing intracranial tumors derived from three different GBM patients were studied. Dynamic and static PET-CT was performed after mice received 100-µCi of FDG intravenously. Tumor-bearing mice were also infused with intravenous D-[U-$^{13}C_6$]-glucose or [U-$^{13}C_5$]-glutamine, then metabolites were extracted from tumors and contralateral brain tissue and analyzed by high-resolution ^{13}C NMR spectroscopy.

Results and Conclusions: FDG-PET demonstrated enhanced glucose uptake in the tumor relative to surrounding brain (Fig 1). As expected, NMR spectroscopy revealed glucose oxidation in the surrounding brain CAC (Fig 2). In the tumors, ^{13}C-labeled lactate was observed in all cases, but all tumors also displayed a fully active CAC (Fig 2). Labeling in glutamate carbon-4 reflected activity of pyruvate dehydrogenase, and labeling in additional glutamate carbons reflected normal oxidative processing of CAC intermediates plus activity of the anaplerotic enzyme pyruvate carboxylase. Overall, the ^{13}C spectra were surprisingly similar between areas of normal brain and areas predominantly composed of GBM cells, differing only by an increased ratio of labeling in glutamine relative to glutamate within the gliomas. Moreover, [U-$^{13}C_5$]-glutamine was taken up by the tumor but not substantially metabolized. The data reveal that tumor metabolism is far more complex than classical models suggest, and that novel targets for therapy and/or imaging may be revealed by probing the metabolism of intact tumors with stable isotopes.

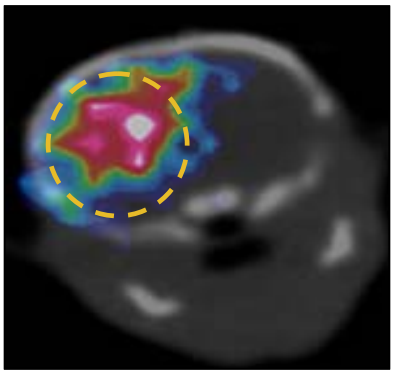

Fig. 1 Brain FDG-PET scan of a tumor-bearing mouse illustrating a prominent uptake of ^{18}F-D-glucose in the tumor (dashed circle) compared to the surrounding brain tissue.

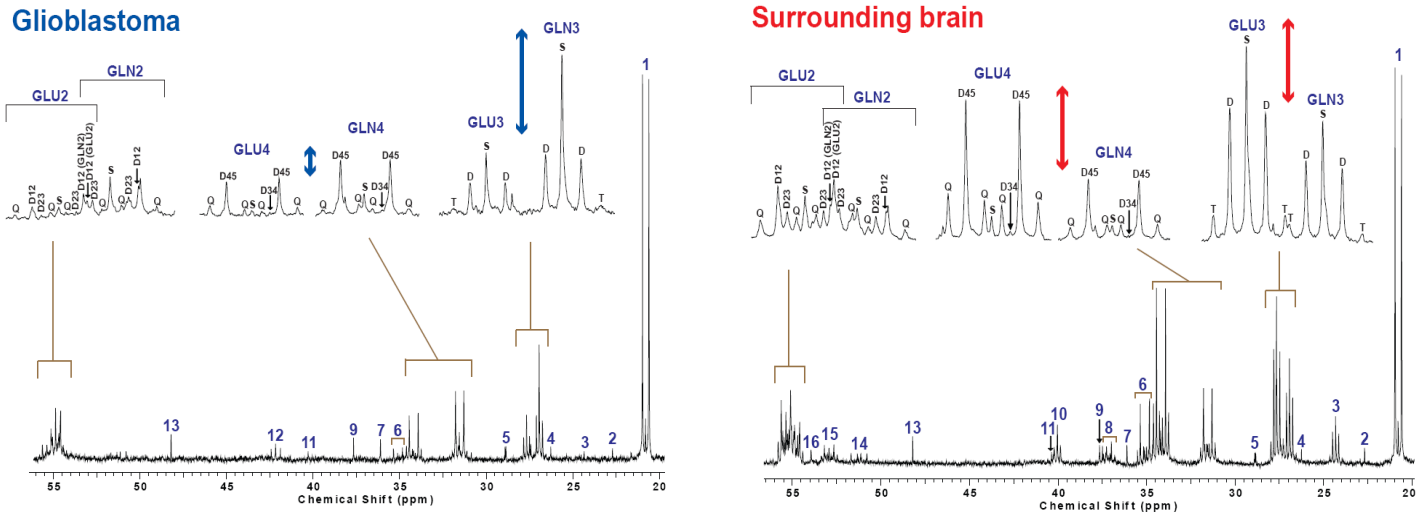

Fig 2. ^{13}C NMR spectra of GBM and surrounding brain from a tumor-bearing mouse infused with [U-$^{13}C_6$]glucose. Insets display the labeling patterns of glutamate (GLU) and glutamine (GLN) in C2, C3 and C4. 1. Lactate C3, 2. NAA6, 3. GABA C3, 4. unassigned, 5. unassigned, 6. GABA C2, 7. Taurine C2, 8. Aspartate C3, 9. Creatine C2, 10. GABA C4, 11. NAA C3, 12. Glycine C2, 13. Taurine C1, 14. Alanine C2, 15. Aspartate C2, NAA C2. Sx: singlet Dxx: doublet, T: triplet, Q: quartet