Alternative Pathways of Glucose Metabolism in a Mouse Model of Human Brain Tumors

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Introduction: In brain, glucose is metabolized primarily through glycolysis and the citric acid cycle to provide energy, and a small fraction (<5%) is metabolized through the pentose phosphate pathway (PPP). In cancer cells, increased flux through the pentose phosphate pathway (PPP) is required to support the high demand synthesis of lipids and DNA. Glycolysis, however, still appears the main glucose oxidative pathway in the cytoplasm. Little is known about the relative activities of both pathways in human malignant brain tumors.

Aims and Methods: [1,2-$^{13}C_2$]glucose generates, through glycolysis, unlabeled lactate and [2,3-$^{13}C_2$]lactate. [3-^{13}C] lactate under this condition is derived either from the PPP or from background natural abundance ^{13}C but not glycolysis. If flux into lactate from [1,2-$^{13}C_2$]glucose is increased, then background natural abundance signal in lactate should decrease. Human glioblastoma (GBM) cells and renal cell carcinoma (RCC) cells were transplanted into mouse brain and allowed to grow for ~6 weeks. [1,2-$^{13}C_2$]glucose was infused intravenously for 150 min. Tumors and their surrounding brains (SB) were removed and analyzed by high-resolution NMR spectroscopy.

Results and Conclusions: Figure 1 illustrates the NMR spectrum of the normal mouse brain. [2,3-$^{13}C_2$]lactate dominated all ^{13}C NMR spectra, confirming high glycolytic capacity in both tumor and non-tumor brain. Increased glycolytic flux of [1,2-$^{13}C_2$]glucose into lactate in tumors should cause a decrease in the ratio [3-^{13}C]lactate / [2,3-$^{13}C_2$]lactate. However, this ratio was not different between tumors and their respective surrounding brains (GBM: 0.20 ± 0.01 vs. surrounding brain: 0.19 ± 0.02; RCC: 0.12 vs. surrounding brain: 0.12 ± 0.03) (Fig. 2). These results suggest that much of the lactate in both tumors exchanges very slowly with glycolytic intermediates or the flux into PPP must be up-regulated in proportion to glycolysis. Unexpectedly, ^{13}C-^{13}C coupling was observed in molecules derived from CAC intermediates in both tumors demonstrating oxidation of exogenous glucose in mitochondria of the tumor. The ^{13}C multiplets in GABA C2 of RCC displayed a lower singlet-to-doublet ratio (S/D12= 0.17) relative to its precursor glutamate C4 (S/D45= 0.34) (Fig. 3). Additionally, *67* kDa isoform of glutamate decarboxylase (GAD67) was highly expressed in this tumor compared to GBM. Both features suggest a substantial and compartmentalized synthesis of GABA in RCC, which can potentially elucidate a relevant role of the GABAergic system in its pathophysiology and, as observer in other tumor types, can be a novel target for the treatment and prevention of this tumor.

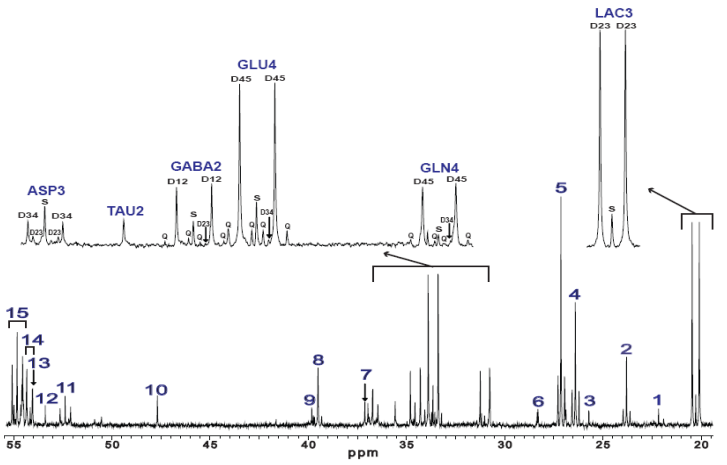

Fig 1. Section of the forebrain spectrum from a non-tumor bearing mouse infused with [1,2-13C2]-glucose. Insets display labeling patterns of lactate C3 and from aspartate C3 to glutamine C4. GLU: glutamate, GLN: glutamine, ASP: aspartate, LAC: lactate, TAU: taurine. 1: N-acetylaspartate C6, 2: GABA C3, 3: unassigned, 4: glutamine C3, 5: glutamate C3, 6: unassigned, 7: creatine C2, 8: GABA C4, 9: N-acetylaspartate C3, 10: taurine C1, 11: aspartate C2, 12: N-acetylaspartate C2, 13: creatine C4, 14: glutamine C2, 15: glutamate C2. S: singlet, Dxx: doublet, Q: quartet.

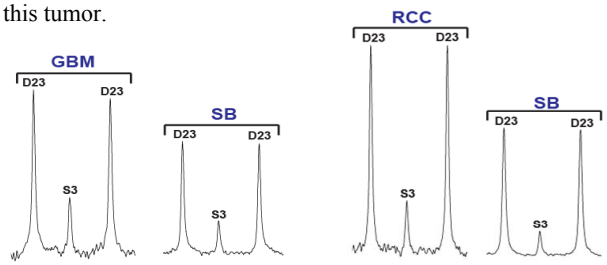

Fig 2. ^{13}C-NMR signal of lactate C3 from both glioblastoma (GBM) and renal cell carcinoma (RCC) and their respective surrounding brains (SB). The S3/D23 ratio of lactate C3, commonly used to estimate PPP vs. glycolysis activity, was not significantly different between the tumors and their respective surrounding brains. S: singlet, Dxx: doublet, Q: quartet.

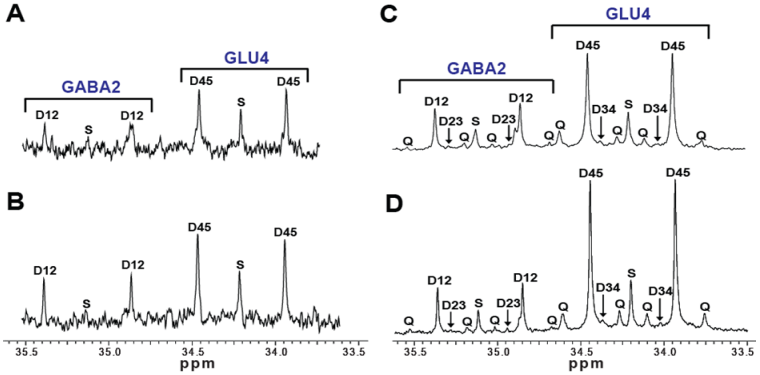

Fig 3. ^{13}C-NMR spectrum illustrating GABA C2 and glutamate C4 labeling patterns in GBM (A), RCC (B) and their respective surrounding brains (C and D). ^{13}C-^{13}C coupling in both isotopomers denotes CAC activity in tumor and non-tumor tissue. Conversely to GBM and brain tissues, GABA C2 does not preserve the labeling pattern of glutamate C4 in RCC (GABA S/D12 ratio = 0.17 vs. Glutamate S/D45 ratio=0.34), suggesting compartmentalization of GABA synthesis in the RCC tumor. S: singlet, Dxx: doublet, Q: quartet.