Detection of γ-glutamyl-transferase activity up-regulation in orthotopic glioma using hyperpolarized γ-glutamyl-[1-^{13}C]glycine

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ABSTRACT

INTRODUCTION: Glutathione (GSH) is often upregulated in cancer cells, where it serves to mitigate oxidative stress. γ-Glutamyl-transferase (GGT) is a key enzyme in GSH homeostasis[1] and is localized on the outer cell membrane. GGT expression, compared to normal brain, is elevated in tumors, including primary glioblastoma[2]. Thus GGT is an attractive imaging target for non-invasive detection of glioblastoma. A recent study reported γ-glutamyl-[1-^{13}C]glycine (γ-Glu-[1-^{13}C]Gly) as a new hyperpolarized agent to probe GGT activity[3]. However, the ability of hyperpolarized (HP) γ-Glu-[1-^{13}C]Gly to monitor redox in an orthotopic glioblastoma model has not yet been evaluated. The goal of our study was to assess the value of HP γ-Glu-[1-^{13}C]Gly for non-invasive imaging of glioblastoma burden.

METHODS: In vivo studies were performed in athymic nude rats bearing orthotopic U87 glioblastoma tumors[4], or tumor-free healthy controls. Dynamic ^{13}C studies were performed by injecting HP γ-Glu-[1-^{13}C]Gly and acquiring dynamic ^{13}C data on a preclinical 3T system. Data were acquired using a slice selective (15mm) flyback spectral-spatial (SPSP) sequence or a SPSP echo planar spectroscopic imaging (EPSI) (5.375x5.375mm^2 in plane resolution). The signal-to-noise (SNR) ratios of the substrate (γ-Glu-[1-^{13}C]Gly) and the product ([1-^{13}C]glycine), as well the product to substrate ratios were evaluated in MestReNova (slice selective) or home-made Matlab code (imaging). The levels of GSH in tumor, contralateral normal-appearing brain and healthy brain tissue, as well as the expression levels of GGT were evaluated using NMR and western blotting respectively. All results are expressed as mean±standart error of mean and $p<0.05$ considered significant.

RESULTS AND DISCUSSION: The enhancement and T1 of HP γ-Glu-[1-^{13}C]Gly were consistent with previously published values[3] (22.9% and 33±3.5s respectively). In vivo HP acquisitions were performed on tumor-bearing animals

when tumors reached a volume of ~0.25cm^3 (Fig.A). Summed spectra from dynamic acquisitions in control and tumor-bearing animals showed no statistically significant difference in the SNR of HP γ-Glu-[1-^{13}C]Gly (Fig.B,C). Control rats showed low or below detection signal of [1-^{13}C]Gly, whereas [1-^{13}C]Gly was readily detected in all tumor-bearing animals (Fig.B,D). Consistent with the higher levels of HP [1-^{13}C]Gly production in tumor relative to normal brain, the [1-^{13}C]Gly-to-γ-Glu-[1-^{13}C]Gly ratio was significantly higher in tumor-bearing animals relative to controls (Fig.E). Spectroscopic imaging illustrated that γ-Glu-[1-^{13}C]Gly is homogeneously distributed in the brain (Fig.F,G) and the ratio of maximum [1-^{13}C]Gly-to-maximum-γ-Glu-[1-^{13}C]Gly was higher in tumor region compared to normal-appearing brain (Fig.H). Importantly, higher [1-^{13}C]Gly in tumor was associated with higher GSH levels (Fig. I,J) and higher GGT expression (Fig. K) in tumor tissue compared to normal brain.

CONCLUSION: For the first time, this study demonstrates the feasibility of using γ-Glu-[1-^{13}C]Gly to monitor GGT activity metabolism in an orthotopic glioblastoma model. The polarization, T1 levels and absence of toxicity of γ-Glu-[1-^{13}C]Gly combined with the presence of the enzyme on the outer cell membrane, are encouraging for future translation to the clinic where this HP probe could help detect the presence of tumor.

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REFERENCES:
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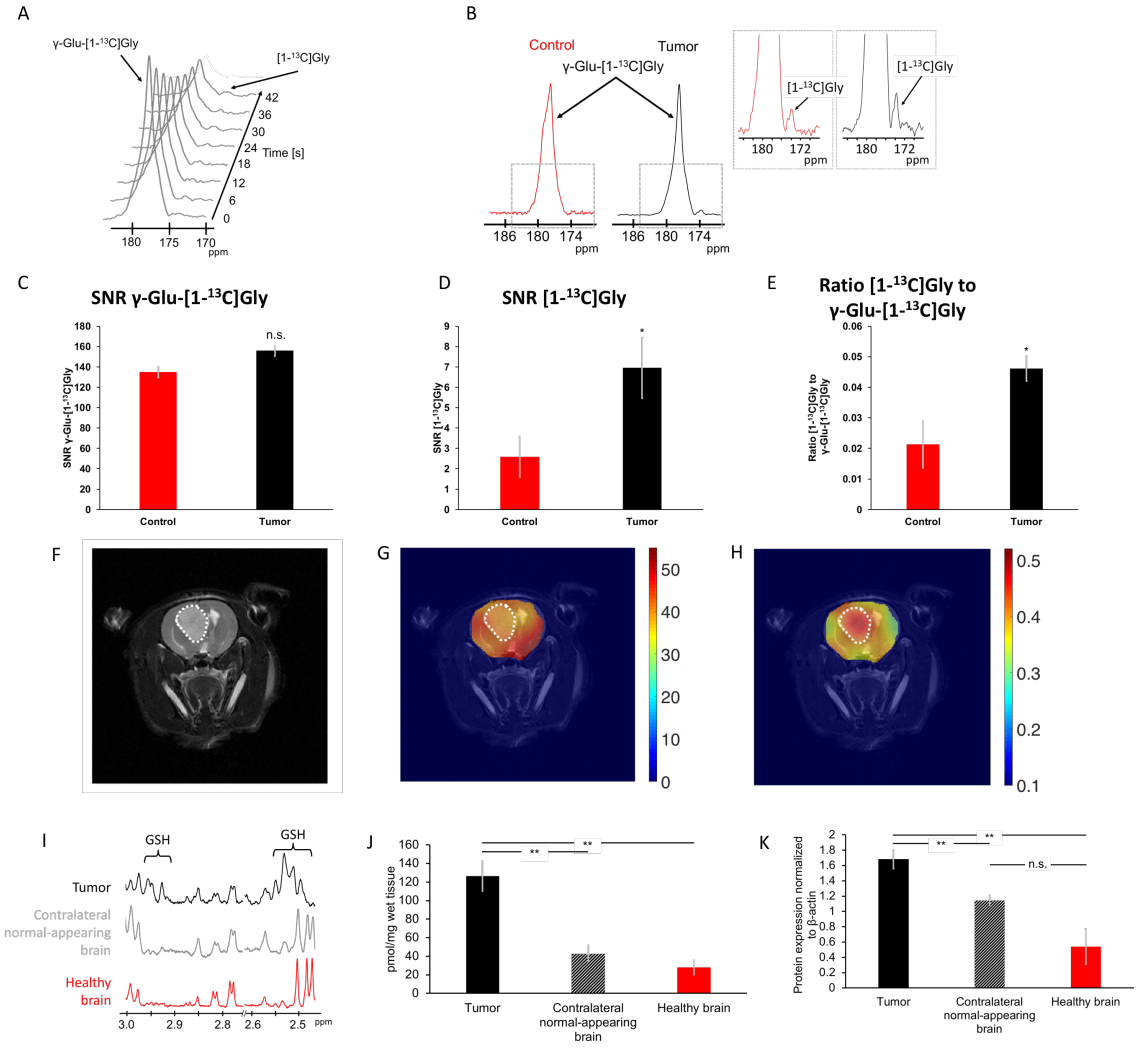
Highlights

γ-glutamyl-transferase (GGT), a key enzyme in glutathione homeostasis regulation, is usually upregulated in glioblastomas, among other tumor types. Here, we demonstrated for the first time the feasibility of using HP γ-glutamyl-[1-^{13}C]glycine to non-invasively monitor the expression of GGT in the brain and in a glioblastoma tumor model.

FIGURE



(A) Stack plot of hyperpolarized ^{13}C data acquired from a tumor-bearing rat, showing decay of HP γ-Glu-[1-^{13}C]Gly and production of HP [1-^{13}C]Gly as a function of time. (B) Sum spectra from the dynamic acquisition and zoom of spectra showing HP [1-^{13}C]Gly production. (C) Quantification of γ-Glu-[1-^{13}C]Gly SNR. (D) Quantification of [1-^{13}C]Gly SNR (E) [1-^{13}C]Gly-to-γ-Glu-[1-^{13}C]Gly ratios. (F) Representative T2-weighted anatomical image from a tumor-bearing animal. (G) Heat map of γ-Glu-[1-^{13}C]Gly. (H) Heat maps of the ratio of maximum [1-^{13}C]Gly to maximum γ-Glu-[1-^{13}C]Gly. White dotted line outlines the tumor. (I) Typical 500MHz ^{1}H MR spectrum of the aqueous face of tumor, contralateral normal-appearing brain and healthy brain with GSH regions highlighted. (J) GSH levels in the 3 tissue groups evaluated by MRS. (K) GGT expression in the 3 tissue groups evaluated by western blot. Black: tumor; Striped black bar: Contralateral normal-appearing brain; Red: Healthy brain. *=p<0.05,**=p<0.01