MR biomarker profile for infiltrative tumor region in malignant glioma

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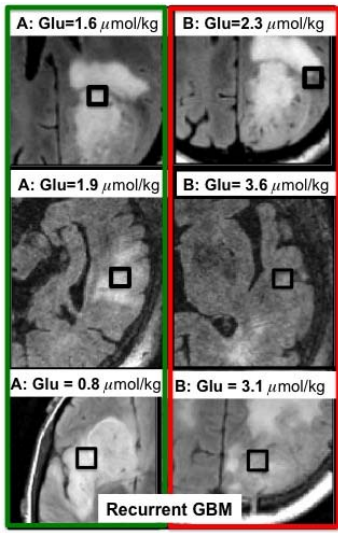
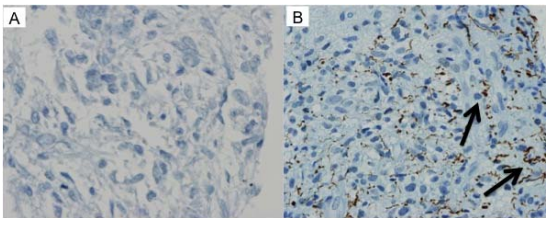
Introduction: MR images are routinely used in brain tumor management and provide an excellent visualization of the global heterogeneity of the tumor. However, since MR intensities have not been linked to biological mechanisms that drive tumor growth and response, they have proven to be inadequate surrogates of tumor presence particularly following treatment. This study will address the inability of MR imaging to accurately define the infiltrating tumor margin, which results in a margin being added to radiotherapy volumes to account for infiltrating cells. Since this volume includes normal cells, radiation dose is reduced. Consequently, gliomas are known to recur within the treatment volume (1) and inclusion of normal brain can impact patient quality of life. The infiltrating tumor region is characterized by complex interactions that are disruptive to brain architecture. Since these regions contain diffusely infiltrating tumor cells, they can have similar cellular density as normal brain. Hence, choline and apparent diffusion coefficient (ADC), typically used to identify tumor regions based on high cellularity, are unlikely to define or locate infiltrative regions. A measure linked with tumor activity and structural disruption is anticipated to provide improved delineation of tumor infiltrative margin. Studies suggest that glioma cells, at the growth margin of an expanding tumor, release glutamate via "system Xc", an electroneutral amino acid transporter that exchanges cystine for glutamate [2] during the synthesis of cellular antioxidant glutathione. Released glutamate kills normal cells via the excitotoxic mechanism [3] and glutathione protects tumors from immune attack and also makes them insensitive to radiation treatment that is mediated by hydroxyl radicals. The goal of this study was to determine if glutamate is an indicator of tumor infiltrating region. Ex-vivo spectroscopy of image guided biopsies was used to obtain glutamate levels from tumor regions and analyzed a) relative to their spatial location within the tumor b) compared with SMI-31 immunostain from its paired tissue sample and c) investigated relative to fractional anisotropy (FA) which is a diffusion imaging measure of structural anisotropy indicative of disrupted brain architecture. SMI-31 is a mouse monoclonal antibody that stains neurofilaments [4] and therefore provides an indication of disrupted neuronal tracts.

Methods Ex-vivo acquisition and analysis: Tissue samples were weighed and placed in custom designed 35 µl zirconium rotors. High-resolution magic angle (HR-MAS) spectroscopy data were acquired at 11.7T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer and 4 mm gHX nanoprobe with the Carr-Purcell-Meiboom-Gill (CPMG) sequence; TR=4s; TE=144ms; 512 scans; 35 minute acquisition, 40000 points, 20000 Hz spectral width. The Electronic Reference to access *in vivo* concentrations (ERETIC) [5] method was used as a quantitation standard. Basis set spectra of 42 metabolites in solution were incorporated into a custom version of the QUEST [6] fitting routine. Concentrations were calculated relative to the peak area of the ERETIC signal. Only concentration estimates within 10% Cramer-Rao error estimate were used for further analysis. Analysis of 22 newly diagnosed untreated GBM and 19 recurrent GBM following treatment samples are reported here. In-vivo analysis: Biopsy locations were recorded by BrainLab (Vector Vision) neuro-navigational system during the surgical procedure relative to the pre-surgical 3T MR images. Fractional anisotropy from within these biopsy locations were obtained from diffusion weighted images and normalized to the median of the whole brain histogram (nFA).

Results and Discussion: **Figure 1** compares the glutamate (Glu) levels from different spatial locations in untreated and recurrent GBM. Glutamate levels from location B at the tumor margin are higher relative to location A at the center of the tumor. Choline and ADC levels were similar between these two locations and consistent with a non-tumor region suggesting that the infiltrating region cannot be defined by increased cellularity. There was no NAA in these regions to indicate the presence of intact axons. Hence the measured glutamate is likely due to release from tumor cells and not associated with normal brain neurotransmission. A significant (p<0.001) positive correlation between glutamate and glycine, which is an indicator of malignancy [7], was also observed in these samples. **Figure 2** compares the SMI-31 immunostain in two samples with different glutamate levels but same tumor cellularity. There is greater SMI-31 immunostain (Figure 2B: arrows) for the sample with lower glutamate relative to the sample with higher glutamate (Figure 2A). Since SMI-31 immunostains for axons, this suggests that there is less axonal damage in regions with lower glutamate. **Figure 3** illustrates the strong inverse relationship (r = -0.33; p < 0.001) between glutamate and normalized fractional anisotropy (nFA) for newly diagnosed and recurrent GBM. This is consistent with the association of glutamate with disruption of normal brain architecture that is reflected in reduced fractional anisotropy. These data also suggest that glutamate maintains its specificity to tumor presence following treatment in recurrent GBM.



Conclusions: Elevated glutamate within tumor regions is associated with structural disruption and axonal damage, which are characteristics of an infiltrative tumor region. Since glutamate is linked with tumor activity, it provides complementary information to morphological measures such as choline, NAA and ADC, which are unable to locate tumor infiltration. Glutamate can be translated for *in-vivo* imaging [8] and used for defining targets for focal therapies. Since glutamate is associated with tumor growth, it is likely to provide an improved assessment of response for cytostatic therapies that target the ability of the tumor to multiply and grow.



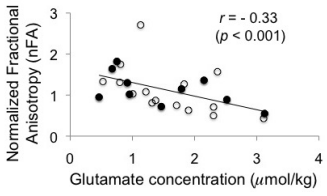


Figure 1: For each patient, glutamate is elevated at location B at the tumor margin relative to location A, more central to the tumor. ADC and choline were similar at both locations and consistent with a non-tumor region, suggesting that infiltrating tumor regions cannot be defined by increased cellularity. Lack of NAA in B indicates that the measured glutamate is released from tumor cells and not from neurotransmission in intact axons. **Figure 2**: High magnification (400x) digitized images of the SMI-31 immunostain from tissue samples with different glutamate levels and the same tumor cellularity. Sample A had higher glutamate levels (Glu = 5.3 µmol/kg) relative to sample B (Glu = 1.90 µmol/kg). Increased preservation of immunostaining for SMI-31 (B: arrows) in the sample with lower glutamate suggests that elevated glutamate in (A) is associated with axonal damage. **Figure 3**: Significant (r = -0.3; p< 0.001) inverse relationship between Glutamate levels and normalized fractional anisotropy (nFA) is observed in data from newly diagnosed (open circles) and recurrent GBM (filled circles). This is consistent with the association of elevated glutamate with disruption of normal brain architecture that is reflected in reduced fractional anisotropy.

References: [1] Chan, 2002 [2] Sontheimer H, 2008 [3] Lipton SA, 1994 [4] Sternberger LA, 1983 [5] Ziarelli, 2006 [6] Ratiney H, 2005 [7] Kinoshita Y, 1997 [8] Srinivasan R, 2006 **Acknowledgements**: NIH grants: RO1: CA127612-01A1 and P01: CA118816-01A2