CEST effect at 2ppm (CEST@2ppm): a potential biomarker for grading brain tumor malignancy

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Introduction:
MRI helps to predict clinical outcome and hence contribute to individualized treatment planning. CEST MRI contrast is due to amine, amide and hydroxyl protons and has been shown to provide imaging maps of metabolites in tissue noninvasively. In this project, we aim to evaluate CEST-MRI for characterization of two intra-cranial tumor models with different growth patterns and malignancy. Unlike dynamic contrast-enhanced MRI used for grading tumor malignancy, CEST imaging doesn't require any exogenous contrast agent and thus may be advantageous in the clinical setting.

Methods:
9L gliosarcoma and F98 glioma cells were implanted into Fisher rat brains according to previous protocols[1,2]. At two different tumor size-matched time-points, tumor-bearing rats (n=5 for each tumor model) were scanned with a 9.4T horizontal bore small-animal MRI scanner using a commercial rat head-coil (35 mm). All animal experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. Z spectra (up to 100 ppm) from the tumor central slice were acquired using 50Hz continuous-wave saturation RF pulse lasting for 3s and single shot Fast Low-Angle SHot imaging (FLASH) readout[1] (shot TR=11.4s and TE=3ms). Total imaging time for 2 averages was about 24 min. Following Z spectra acquisition, images for B_0 and B_1 mapping were acquired[1]. Nonlinear constrained fitting routine "lsqcurvefit" was performed in MATLAB to fit pixel by pixel Z spectral data within ~±10ppm range, normalized by 100ppm and center-corrected using B_0 field map. The flipped Z spectra ($1-M_z/M_0$) were fitted using five Lorentzian functions for Nuclear Overhauser Effect (NOE), magnetic transfer (MT), water, the CEST effect at 2ppm (CEST@2ppm) and amide proton transfer (APT), located at -3.2, -1.5, 0 and 2.0 and 3.6 ppm respectively. Fitting was loosely constrained as amplitude within 0.01 to 10 times of initials, line-width 0.5 to 2 times of initials and chemical shifts vary within 10% of corresponding line-widths. After this first round of fitting, water and MT peaks were subtracted from the entire Z spectra and a 2nd round of fine-tuning fitting of the rest three peaks was performed. Fitting R^2 was calculated pixel by pixel. Following Z spectral acquisition, ^{1}H point resolved spectra (PRESS) were acquired with (256 average) or without (32 average) VAPOR[3] water suppression from localized voxels ($3 \times 1.5 \times 2$ mm^3) located in tumor or normal brain tissue. One-way student's t-test was performed to compare different tumor models or imaging time points. Significant difference was considered when $p<0.05$.

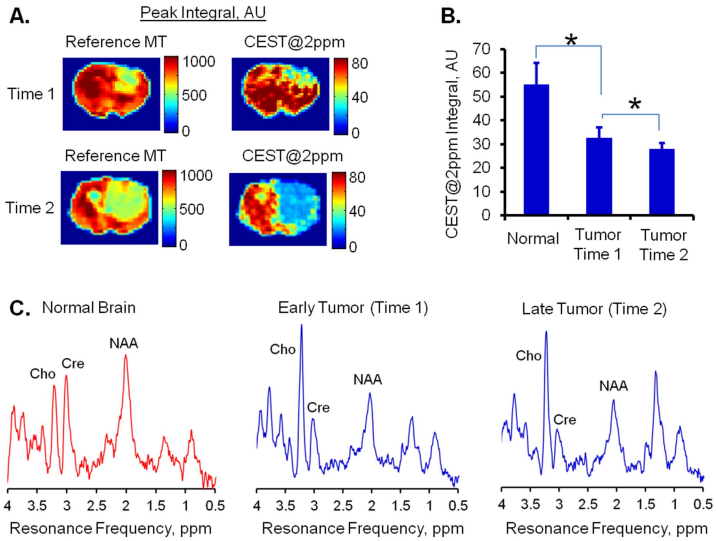

Figure 1. The CEST@2ppm (from water) integral decreases in tumor (9L) compared to normal brain tissue and further reduced as tumor progresses to a later stage (A and B). This correlates with the creatine change detected with MR spectra, which were normalized to corresponding water reference (at 4.7ppm) and summed between animals. AU: Arbitrary Unit.

Results and Discussion:
A total of five visible peaks was found to contribute to Z spectra acquired with low saturation RF magnitude, including direct saturation, MT, APT, NOE and a off-resonance 2ppm peak, consistent to previous reports [4]. Pixel by pixel fitting provides us the integral, chemical shift, line-width and amplitude maps of these contributions to Z spectrum. The CEST@2ppm integral map defines metabolically affected tumor area differently from other conventional MRI contrasts including MT (Figure 1A). APT integral increases while the CEST@2ppm integral decreases in tumor compared to normal brain tissue, indicating that the CEST@2ppm peak may not be mainly contributed from amide protons associated with proteins[5] and lipids[6]. The CEST@2ppm peak was also tentatively assigned to glutamine[5]. However, CEST effect from glutamine fast-exchanging amine protons cannot be detected at physiological pH[1,7].

Among the major brain tumor metabolites with slow exchangeable protons (<600Hz), only creatine exhibits CEST effect centered at +2ppm[8-10] from water that can be detected with low saturation RF amplitude. In addition, the CEST@2ppm integral further decreases as tumor progresses (Figure 1A and B). This is correlated with the creatine concentrations detected using single voxel MR spectroscopy (Figure 1C). Hence we suggest creatine to be the major contribution to the CEST@2ppm, consistent to early studies[11,12]. However, quantitative justification is required to assess the percentage of creatine contribution to the CEST@2ppm.

Creatine has a critical function in the cell energy system and plays a critical role in the regulation of respiration and energy fluxes[13]. Creatine has been shown to decrease in brain tumors due to cancer hypermetabolism[14]. In addition, creatine concentration tends to be even lower in high-grade tumors[15], and such a decrease may play an important role in brain tumor grading. We scanned the aggressive F98 and less aggressive 9L brain tumor. F98 tumors have significant lower CEST@2ppm effect compared to 9L tumors (Figure 2), indicating that it might be feasible to differentiate brain tumor malignancy based on the CEST@2ppm. However, further validation may be necessary in a larger group and testing models with different malignancies.

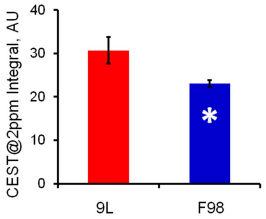

Figure 2. F98 tumors have significantly lower CEST@2ppm compared to 9L ($p<0.05$).

Conclusion:
Creatine may be the major contribution to the CEST@2ppm quantified through fitting Z spectrum with Lorentzian functions. Once validated, the high-resolution creatine mapping method could be used for grading brain tumors.

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