Towards the contrast mechanism of chemical exchange saturation transfer (CEST) in tumors at 9.4T

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Target Audience: Investigators who are interested in the biophysical mechanism of chemical exchange saturation transfer (CEST) imaging in oncology and its use in probing tumor microenvironment.

Purpose: Chemical exchange saturation transfer (CEST) imaging has been shown to have considerable clinical potential as an endogenous molecular imaging technique. Theoretically, amide proton transfer (APT), a specific form of CEST, should be sensitive to protein concentration in tissues (1). However, whether the APT contrast obtained in CEST measurements *in vivo* reflects real differences in protein concentrations between tumors and normal tissues remains to be established. In this study, we correlated APT contrast with multiple other MR parameters and protein concentration in tumors and normal tissues. In additional, nuclear Overhauser effects (NOE) were also investigated as an alternative form of imaging contrast.

Methods: *Animal and cancer model*: Eight F344/Hsd rats were injected with 9L glioma cells in their right brain hemispheres to allow tumors to grow to 30-40mm^3.

In vivo imaging: All experiments were performed on a 9.4T Agilent MRI scanner. CEST images were acquired with continuous wave saturation pulses (1μT for 5 seconds), and all Z-spectra were normalized and corrected for B0 inhomogeneities. Maps of relaxation rates $R_1(=1/T_1)$ and $R_2(=1/T_2)$ were obtained using inversion recovery and spin echo with multiple echo times, respectively. Quantitative magnetization transfer (qMT) using a selective inversion recovery method (2) to map pool size ratio (PSR), and apparent diffusion coefficients (ADC) were estimated from pulse gradient spin echo acquisitions. All images were obtained using a 2-shot echo-planar imaging sequence with 333μm in-plane resolution. The conventional APT contrast, MTR_{asym}, was obtained by subtraction of down-field from up-field spectra. In addition, an alternative approach that avoids macromolecular asymmetry effects and uses linear fitting of up-field spectra only (3) was implemented to characterize amide content and exchange, labeled APT*. Similar methods were also performed to analyze NOE effects, except that the spectra were fit to 3rd-order polynomials.

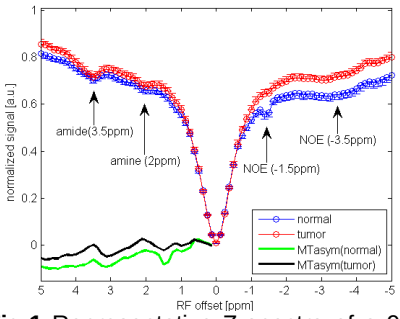

Fig.1 Representative Z-spectra of a 9L glioma tumor (red) and contralateral normal tissue (blue). Note that an NOE dip at -1.5ppm was observed in normal tissues but not in tumors.

Extractable protein determination: After imaging, rats were decollated with a guillotine and then frozen in a hexane/dry ice bath. The whole procedure took < 2 minutes to prevent protein degradation. Frozen brains were dissected at -20ºC, and tumors and contralateral normal tissues were cut out. Total extractable (mainly soluble) proteins were extracted with 2% of SDS, and their concentrations were determined by the Bradford method (4).

Results and Discussion: Fig.1 shows representative Z-spectra of a 9L glioma tumor and contralateral normal tissue. There is a clear APT effect at 3.5ppm which was significantly larger in tumors. Interestingly, a distinct NOE peak was also found at -1.5ppm in contralateral normal tissues which vanished in the tumor. Fig.2 shows pixel-wise correlations of four CEST parameters (MTR_{asym}, APT*, NOE(-1.5ppm) and NOE(-3.5ppm)) with R_1, R_2, PSR and ADC. Tumors show a significant decrease in R_1 and PSR, which indicates a *decreased* total protein and macromolecular concentration in tumors, consistent with an extensive earlier literature. There is also an increase in ADC, suggesting a lower cellularity in tumors. By contrast, R_2 was higher in tumors, which differs from results obtained at lower field strength, consistent with an increased chemical exchange contribution to R_2 at higher field. For CEST measurements, both MTR_{asym} and APT* were higher in tumors. The biochemical protein determination found that tumors had only slightly higher total extractable proteins compared to contralateral normal brain tissue, but the differences were insufficient to explain the much larger differences in other MR parameters. Interestingly, NOE(-1.5ppm) shows a very clear contrast to differentiate tumors from contralateral normal brain tissues.

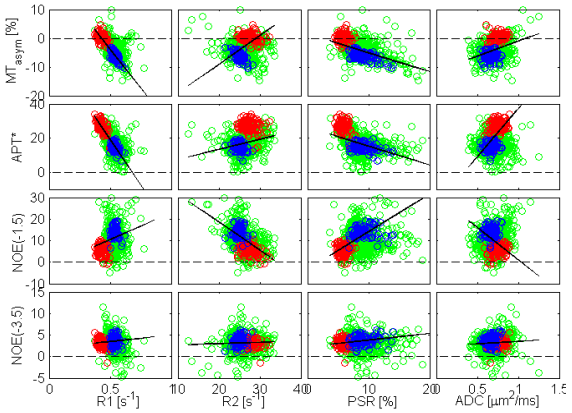

Fig.2 Representative correlations between CEST parameters (MTR_{asym}(3.5ppm), APT(3.5ppm), NOE(-1.5ppm), NOE(-3.5ppm)) and multiple MR parameters (R_1, R_2, PSR, and ADC). Red points represent tumor, blue represent contralateral normal tissue, and green for all other rat brain tissues.

Conclusion: The differences in R1 and PSR confirm that the total macromolecular content relevant for affecting water relaxation is lower in tumors than in normal tissue, whereas APT and R2 detect other variations in composition that cause an increased contribution from exchanging protons. The interesting NOE(-1.5ppm) may provide a new imaging parameter to detect cancer, and the underlying biophysical mechanism is under investigation.

References: (1) van Zijl. MRM 2011 (2) Gochberg. MRM 2003 (3) Jin. MRM *in press* (4) Bradford. Anal. Biochem. 1976