Molecular CEST imaging of underglycosylated MUC-1 expression

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INTRODUCTION

Mucin-1 is a marker of epithelial cell lines that is expressed in an underglycosylated form (uMUC-1) in neoplastic cells derived from both epithelial and non-epithelial cell types. The specificity of this marker for early tumorigenesis makes it a target of great interest for molecular imaging[1]. Chemical Exchange Saturation Transfer (CEST) MRI is a promising molecular imaging modality that can amplify chemical shift signals of specific chemical functional groups based on exchange of their protons with water[2,3]. Underglycosylation of mucin implies that its hydroxyl content would be much reduced compared to normally glycosylated tissue. MUC-1, with a core protein mass of 120-225 kDa, increasing to 250-500 kDa with glycosylation, extends no less than 500 nm beyond the surface of the cell[4]. We hypothesized that a relative difference in uMUC-1 expression may therefore be readily detected with CEST MRI through changes at characteristic chemical shifts for hydroxyl groups.

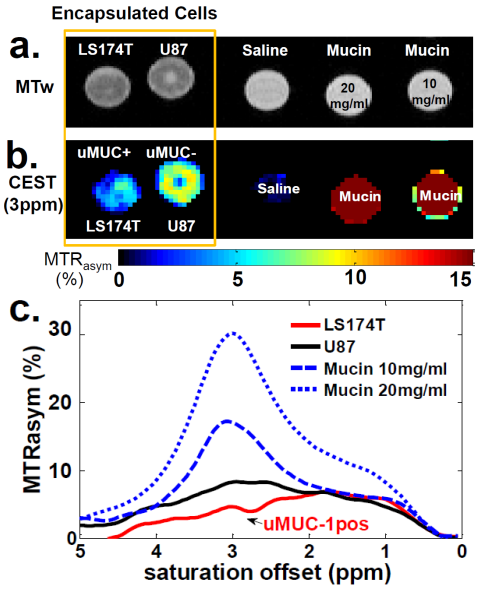

Figure 1. *In vitro* CEST images and spectrum for encapsulated LS174T (uMUC-1^{+}) and U87 (uMUC-1^{-}) cells.

METHODS

Encapsulated cells: LS174T (uMUC-1 positive, i.e., underglycosylated) and U87 (uMUC-1 negative, i.e., heavily glycosylated) tumor cells were encapsulated in Alginate-PLL-Alginate microcapsules[5] at ~1000 cells/capsule. Microcapsules suspended in 0.9% saline were loaded into 1 mm capillaries. Reference standards contained mucin from porcine stomach (Sigma, M2378) dissolved in 0.9% saline.

Animal studies: CB17-PRKDSCID/NCR female mice (6-8 weeks) were inoculated with 1.5x10^{5} of LS174T and U87 cells in each cerebral hemisphere at 1 mm anterior, 2 mm lateral, and 2.5 mm ventral to bregma. CEST MRI was performed at 15-21 days post injection.

Image Acquisition and Analysis: Encapsulated cells were imaged using a Bruker 500MHz 11.7T vertical scanner and *in vivo* experiments were performed on a Bruker 9.4T scanner with a modified RARE sequence: slice thickness=1 mm, matrix size=96x48, FOV=1.15 cm x 0.55 cm for the *in vitro* and 1.7 cm x 1.6 cm for the *in vivo* studies, CW saturation pulse=3 sec, RARE factor=8, frequency range = -5 pm to 5 ppm (0.2 ppm increment) for encapsulated cells and -4.5 ppm to 4.5 ppm (0.3 ppm increment) *in vivo*, TR/TE= 6000 ms/14.05 ms for encapsulated cells and 5000 ms/11.72 ms *in vivo*, and NA=2. B_0 inhomogeneity was corrected using WASSR[6] with saturation pulse = 0.5 uT/50ms, and frequency range = -1 ppm to 1 ppm (0.1 ppm increment). Z-spectra were calculated from sample ROIs after B_0 correction for each voxel using WASSR. $MTR_{Asymmetry}$= 100%*($S^{-\Delta\omega}$–$S^{+\Delta\omega}$)/S^{0} was computed for each offset Δω.

RESULTS AND DISCUSSION

Encapsulated cells showed differential CEST contrast depending on uMUC-1 expression, with the largest differences in CEST MTR_{asym} spectrum between 2 and 4 ppm offset from water, in agreement with the measured contrast of isolated Mucin-1 (**Figure 1**). Similarly, differential CEST contrast was observed *in vivo* based on uMUC-1 expression, with uMUC-1 expressing cells showing a lower CEST contrast between 0.5 and 2 ppm (**Figure 2).** The specific reduction of CEST contrast for LS174T cells is likely due to the different uMUC-1 glycosylation levels. The different chemical shifts of the maximal effects between the *in vitro* and *in vivo* preparations may be due to different pH buffering and also back-exchange effects to other protons such as amide.

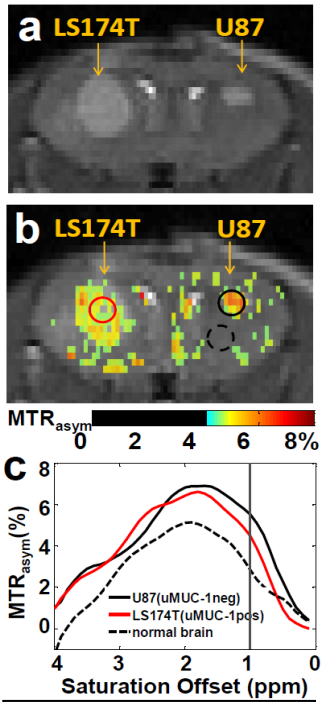

Figure 2. *In vivo* mouse brain MTw image (**a**), CEST image at 1 ppm (**b**), and CEST MTR_{asym} spectrum (**c**).

CONCLUSIONS

Cell lines with differential expression of uMUC-1 show differential CEST contrast both *in vitro* and *in vivo*. The reduction of CEST contrast with uMUC-1 expression is likely due to the reduced number of hydroxyl groups as compared to heavy glycosylation. This negative contrast can potentially be used to non-invasively phenotype tumors and detect early tumorigenesis based on uMUC-1 expression.

REFERENCES

1) A. Moore et al., Cancer Res. 64:1821 (2004). 2) P.C.M. van Zijl et al., PNAS. 104:4359 (2007). 3) J. Zhou et al., Nat. Med. 17:130 (2011). 4) M. Brayman et al., Reprod. Biol. Endocrinol. 2:4 (2004). 5) B.P. Barnett et al., Nat. Prot. 6:1142 (2011). 6) M. Kim, et al. MRM 61:1441 (2009).