Molecular CEST Imaging of Mucins with Different Glycosylation Levels

Xiaolei Song[1,2], Raag D Airan[1,2], Dian R Arifin[1,2], Amnon Bar-Shir[1,2], Lea Miranda[1,2], Deepak Kadayakkara[1,2], Guanshu Liu[1,3], Assaf A. Gilad[1,2], Peter C.M. van Zijl[1,3], Michael T. McMahon[1,3], and Jeff W.M. Bulte[1,2]

[1]*Division of MR Research, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University, Baltimore, MD, United States,* [2]*Cellular Imaging Section, Institute for Cell Engineering, The Johns Hopkins University, Baltimore, MD, United States,* [3]*F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States*

Target Audience: Researchers working in the field of cancer molecular imaging, protein glycosylation and CEST/MT imaging.

Purpose:

Tumor-associated glycosylation changes have been observed for decades[1-2], and are associated with tumor proliferation, metastasis, and angiogenesis. Cell-surface glycoproteins including mucins, in particular MUC-1[2-3], have been used as novel diagnostic and therapeutic targets. In many adenocarcinomas (e.g. colon, breast, and ovarian cancers), MUC-1 is overexpressed in aberrant forms generating an underglycosylated MUC-1 (uMUC-1) antigen (**Fig. 1**). Chemical Exchange Saturation Transfer (CEST) MRI is a molecular imaging modality that can amplify signals from specific functional groups in proteins, peptides, and sugars based on the exchange of their protons with water[4]. Owing to the abundance of both MUC-1 and its exchangeable protons on attached glycans (-OH) and core protein (-NH,-NH_2), the mucin shows a characteristic CEST contrast from 0.5 ppm to 4 ppm[5]. **We aimed to test whether CEST MRI is able to detect changes in glycosylation levels of mucins, and to differentiate uMUC-1 positive tumor cells (expressing underglycosylated MUC-1) from uMUC-1 negative cells (expressing normally glycosylated MUC-1).**

Methods:

Deglycosylation of mucin: Due to the complicated O-linked glycosylation of mucin, chemical deglycosylation is preferred over enzymatic methods[6]. The oligosaccharide chains on porcine stomach mucin (Sigma, M-2378) were removed using anhydrous trifluoromethanesulfonic acid (TFMS) treatment. Both deglycosylated and untreated mucin were dialyzed against water, lyophilized and dissolved at a conc. of 4.0 mg/ml in PBS (pH=7.1) for imaging.

Encapsulated cells: Three cell lines (MCF10A, non-tumorigenic human breast carcinoma; and LS174T and HT29, both human colon carcinomas) with different MUC-1 glycosylation levels were encapsulated in alginate-PLL-alginate microcapsules[7] at 1000 cells/capsule in order to minimize cell sedimentation and variations in cell density.

Image acquisition and analysis: Images were taken on a Bruker 11.7T scanner, using a RARE sequence with CW saturation pulse of B_1=3.6μT, T_{sat}=3 s and frequency incremented every 0.2 ppm from -6 to 6 ppm for phantoms and every 0.25 ppm from -5 to 5 ppm for cells; TR=6 s, effective TE=17-19 ms, matrix size=96x64. CEST contrast was quantified by $MTR_{asym} = (S_{-\Delta\omega}-S_{+\Delta\omega})/S_0$ after a voxel-by-voxel B_0 correction, with characterized mean Z-spectra and MTR_{asym} spectra for sample ROIs plotted.

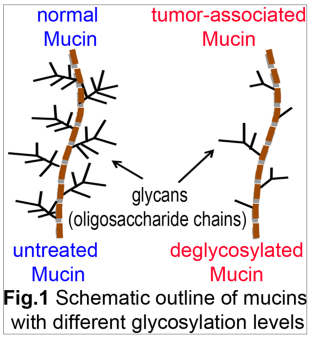

Fig.1 Schematic outline of mucins with different glycosylation levels

Results:

The untreated and deglycosylated mucin could be easily differentiated in both Z-spectra and MTR_{asym} spectra (**Fig. 2a,b**), with a significant reduction of CEST contrast over a broad chemical shift range, i.e. ~80% reduction from 0.5 to 2 ppm and ~50% loss from 2 to 4 ppm respectively. **Fig.2c** is a MTR_{asym} contrast map at 1.8ppm peak. The deglycosylation was confirmed by SDS-PAGE electrophoresis (**Fig.2d**), where deglycosylated mucin showed a MW of 70-100kD, whereas untreated mucin did not show any bands due to the MW being >260kD[8]. We then compared the CEST contrast produced by 3 cell lines with different MUC-1 expression: LS174T and HT29, both expressing underglycosylated MUC-1 (i.e., "uMUC-1 positive"), and MCF10A, expressing normally glycosylated MUC-1 (i.e. "uMUC-1 negative)". Both the MTR_{asym} spectra (**Fig. 3c**) and contrast maps (**Fig. 3d,e**) clearly show that the underglycosylated MUC-1 tumor cell lines (LS174T and HT29) have a lower CEST contrast from 2 to 4 ppm. The MUC-1 glycosylation level was validated by immunostaining with an antibody detecting normally glycosylated MUC-1 (anti-MUC1 antibody, Epitomics) with red=MUC-1, blue=nuclei (DAPI) (**Fig 3f**).

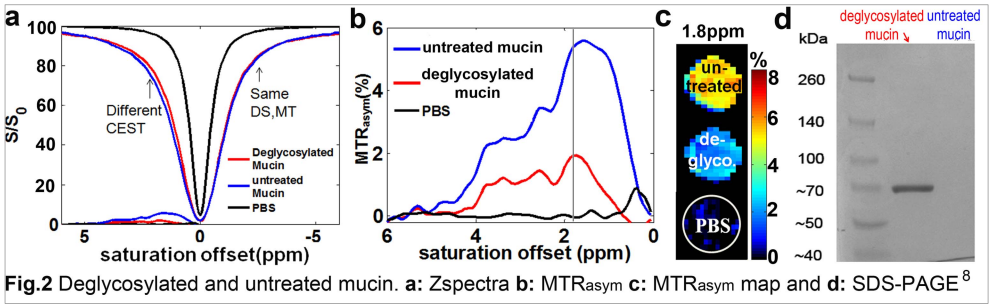

Fig.2 Deglycosylated and untreated mucin. **a:** Zspectra **b:** MTR_{asym} **c:** MTR_{asym} map and **d:** SDS-PAGE[8]

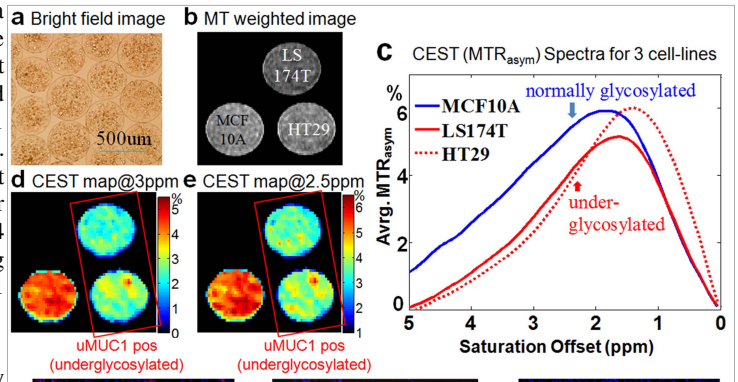

Fig. 3 Microscopy, MTw image, CEST spectra and images, and immuno-staining of encapsulated cell lines with different MUC-1 glycosylation levels.

Discussion and Conclusion:

Deglycosylated and untreated mucin proteins could be easily differentiated by CEST MRI *in vitro*, with the deglycosylated sample showing >80% reduction in -OH peak. The MUC-1 cancer marker also exhibits differential CEST contrast between 0.5 and 4 ppm depending on the glycosylation levels, which is lower for the two cell lines having underglycosylated MUC-1. Our results suggest that CEST imaging of MUC-1 may potentially be used as a surrogate marker to non-invasively assess tumor malignancy and tumor progression.

References: [1]Hakomori, PNAS 99:16 (2002). [2]Hollingsworth, Nat. Rev. 4: (2004). [3]Moore et al., Cancer Res. 64: (2004). [4]van Zijl et al., PNAS. 104:(2007). [5]Song et al, Proc.ISMRM 2334: (2012). [6]Edge, Biochem J. 376:2 (2003). [7]Barnett et al., Nat. Prot. 6: (2011). [8]Piel et al. Reprod. Nutr. Dev. 44 (2004). Supported by NIH grants R01EB015031 and R01EB015032.