Oxidative stress sensitive magnetization transfer MRI of prostate cancer

Rongwen Tain[1,2], Michael Abern[3], Karen Xie[1], X. Joe Zhou[1,2], and Kejia Cai[1,2]
[1]*Radiology, College of Medicine, University of Illinois at Chicago, Chicago, Illinois, United States,* [2]*Center for MR Research, College of Medicine, University of Illinois at Chicago, Chicago, Illinois, United States,* [3]*Urology, College of Medicine, University of Illinois at Chicago, Chicago, Illinois, United States*

Target audience: Scientists or clinicians who are interested in cancer metabolism, oxidative stress and magnetization transfer (MT).

Purpose: The disturbed balance of the cellular redox state due to oxidative stress may occur in many pathologic states including cancer, heart diseases, neurodegenerative diseases, and diabetes. Developing imaging biomarkers for oxidative stress is a key research area[1-3]. Tumor redox state heterogeneity revealed by the optical redox scanning of *ex vivo* tissues has been found to be associated with tumor aggressiveness[4,5]. However, the redox scanning is invasive, limiting its use. Recently, we presented the first non-invasive MR imaging method for mapping the tissue redox state based on the endogenous Chemical Exchange Saturation Transfer (CEST) contrast[6]. Endogenous CEST contrast from *in vivo* breast tumors was found to be significantly proportional to cancer redox ratio measured with optical scanning[6] (**Fig. 1A**). It is well known that magnetization transfer can occur via chemical exchange (CEST) and/or dipole-dipole interactions (Nuclear Overhauser Enhancement or NOE). The purpose of this study is to investigate if MT in the broad definition is sensitive to oxidative stress.

Methods: All *in vivo* animal studies were performed according to an Institutional Animal Care and Use Committee approved protocol. Athymic nude mice bearing DU-145 and PC-3 prostate tumor xenografts (n=3 each, 5 weeks post implantation) were scanned at a Varian horizontal 9.4T MRI scanner. CEST z-spectra were collected from tumor central cross-sections using a custom sequence with a frequency selective rectangle saturation pulse (B_1=250 Hz, 1 s) followed by Fast Low-Angle Shot (FLASH) readout[7]. Other parameters were: flip angle = 15°, readout TR/TE = 6.2/2.9 ms. In addition, MT 'on' and 'off' images at +20 and +100 ppm were acquired for the calculation of the MT ratio (MTR). After MRI, snap-frozen tumors were embedded for redox fluorescence imaging[4,5]. Histograms of the MTR contrast and NADH redox ratio from each tumor was fit with two Gaussian distributions to separate the generally more oxidized tumor core and less oxidized rim[6]. Tumor core and rim values were pooled together for the correlation analysis between redox ratio and MTR. In a controlled study to prove the modulation of MT contrast by oxidative stress, CEST Z spectra of fresh egg white tissue treated with H_2O_2 (2.5% v/v for 1 hour, room temperature) or equivalent PBS were collected in the similar means except for using 3s 50Hz saturation pulse.

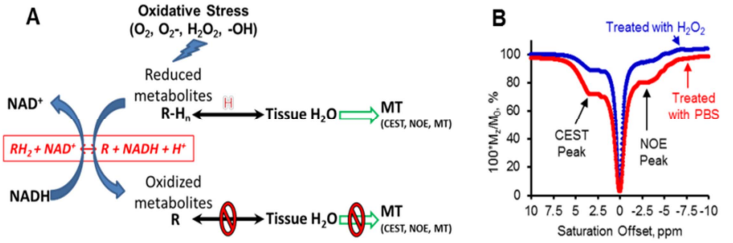

Fig. 1. The fundamental link between tissue redox and MT contrast (A). Under excessive oxidative stress, metabolites (R-H_n, n=1-3), such as small molecules, peptides, proteins, lipids and DNAs, can be oxidized (R) and lose protons that may exchange or interact with bulk water to produce MT contrast. Demonstrated in Z-spectra (B) that H_2O_2 greatly reduces CEST, NOE and the semi-solid MT contrast from fresh egg white tissue.

Results: Treatment with H_2O_2 greatly changed the appearance of Z spectra (**Fig. 1B**) showing distinctive reductions in the NOE, CEST and the semi-solid MT contrasts in egg white tissues. Negligible changes were observed in pH, T_1 and T_2 due to H_2O_2. *In vivo* Z spectra from prostate tumors showed reduced overall MT contrast from the DU-145 tumors comparing to the PC-3 tumors (**Fig. 2A**). Their MTR maps show similar patterns as the NADH redox ratio maps measured with the *ex vivo* optical redox scanning (**Fig. 2B-C**). The histograms of tumor MTR and redox ratio were fit with two Gaussian distributions, which were used to evaluate tumor core to rim heterogeneity (**Fig. 3A**). The MTR was found to be significantly correlated with NADH redox ratio (Fig. 3B), consistent with the *ex vivo* egg white experiments (**Fig. 1B**).

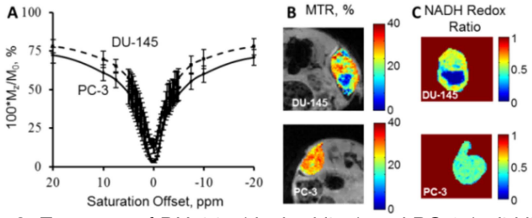

Fig. 2. Z-spectra of DU-145 (dashed line) and PC-3 (solid line) prostate tumors (A). Data were averaged within entire tumor and across all tumors for each tumor line. MTR (B) and NADH redox ratio (C) maps of a typical DU-145 tumor (top) and a typical PC-3 tumor (bottom).

Discussion: Supported by *ex vivo* and *in vivo* experiments, this study further validates and strengthens the novel concept that the oxidative stress can be reflected in the magnetization transfer contrast. The correlation between the MT MRI and the NADH redox ratio indicates that *in vivo* MT contrast may serve as an imaging biomarker for tissue oxidative stress. It is worth noting that the MT contrast *in vivo* can be affected by many other factors. Thus, MT MRI for quantitative redox state characterization may require calibration and the comparison may be restricted within similar tissues. Despite these limitations, non-invasive MT MRI for tissue oxidative stress may impact a broad range of basic and clinical studies.

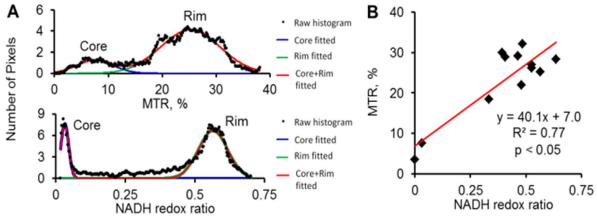

Fig. 3. Demonstration of the Gaussian-fitting analysis of the MTR and NADH redox ratio histograms (A). The CEST contrast and the redox indices from the core or rim of individual tumors were significantly correlated (B, p<0.05).

Acknowledgement: The Authors sincerely acknowledge the Chance lab at the University of Pennsylvania for the service of redox scanning. **References**: [1] Cook JA, et al. Seminars in Radiation Oncology. 2004;14:259-66. [2] Barnham KJ, et al. Nat Rev Drug Discov. 2004;3:205-14. [3] Allen RG, et al. Free Radic Biol Med. 2000;28:463-99. [4] Li LZ, et al. PNAS. 2009;106:6608-13. [5] Xu HN, et al. Journal of biomedical optics. 2010;15:036010. [6] Cai K, et al., Mol Imaging Biol., 2014 16(5): 670-9. [7] Cai K, et al., Nat Med. 2012;18:302-6.