Imaging of Prostate Cancer Metabolic Heterogeneity with CEST and MT MRI

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Introduction: Metastasis is the primary cause of death of prostate cancer patients. Predicting prostate tumor aggressiveness (metastatic potential) to assist treatment strategies is of high importance. Previously, mitochondrial redox scanning (cryogenic NADH/Fp (reduced nicotinamide adenine dinucleotide/oxidized flavoproteins) fluorescence imaging) of *ex vivo* tissues has been used to reveal tumor heterogeneity (core-rim pattern) which is found to be highly correlated to tumor aggressiveness in breast cancer and melanoma mouse xenografts [1, 2]. A non-invasive MRI method could serve as a surrogate metabolic imaging biomarkers for tumor metastatic potential. Magnetization transfer (MT) contrast is widely used for quantifying macromolecular concentration based on slow exchangeable protons with <30Hz exchange rate, while chemical exchange saturation transfer (CEST) contrast using high magnitude of saturating RF magnitudes can be used to map free amino acids with fast exchange rate (typically >1 kHz), such as Glutamate [3]. Tissue levels of macromolecules and amino acids may be related to tumor mitochondrial redox state by amino acid metabolism and protein biosynthesis. In this study, MT and CEST MRI techniques have been used to characterize prostate tumor heterogeneity and correlate with redox scanning of prostate tumor xenografts in order to identify imaging biomarkers for metastatic potential.

Methods: Two classical cell lines of human prostate cancer, PC-3 and DU-145 were chosen for the initial experiments, which have high and moderate invasive potential, respectively [4]. Athymic nude mice bearing DU-145 (n=3) and PC-3 (n=4) prostate tumors (5 weeks growth period, tumor size 207.0 ± 66.6 mm^3 and 114.5 ± 35.1 mm^3 respectively) were scanned with a Varian 9.4-T horizontal MRI scanner. CEST Z-spectra from -5 to 5 ppm were collected from tumor central cross-sections using a custom-programmed sequence, with a frequency selective rectangle saturation pulse (B_1=250 Hz, 1 s), followed by a segmented RF spoiled gradient echo readout. Sequence parameters were: field of view 35×35 mm^2, slice thickness 2 mm, flip angle 15°, readout TR 6.2 ms, TE 2.9 ms, matrix size 128×128, and number of averages 2. One saturation pulse followed with 64 segment acquisition was repeated every 4 second. B_0 and B_1 maps were generated for correcting CEST contrast [3,5]. In addition, MT 'on' and 'off' images with +20 and +100 ppm saturation offsets were acquired. For redox scanning, mice under anesthesia underwent snap-freezing procedures and tumors were harvested and embedded for redox scanning [1,2]. All animal experiments were performed according to a protocol approved by Institutional Animal Care and Use Committee.

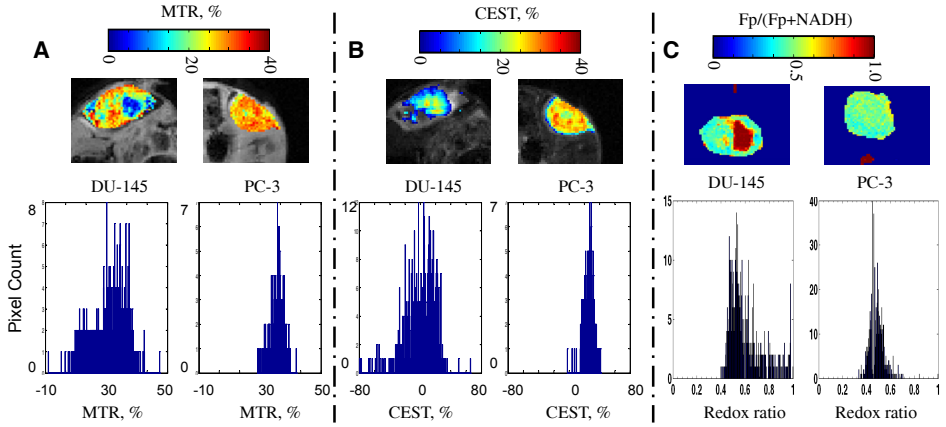

Figure 1. MTR (A), CEST (B) and redox ratio (C) maps of a representative DU-145 and PC-3 tumor. These methods appeared to be consistent with all showing higher level of heterogeneity in DU-145 than PC-3 shown from the corresponding histograms. CEST map shows small ring-core heterogeneity in PC-3 which is not visible from the redox ratio map probably due to the slight difference in tissue depth.

Results: DU-145 tumors showed heterogeneous MT, CEST and redox ratio (Fp/(Fp+NADH)) contrasts while PC-3 maps were relatively homogeneous (Figure 1). Extremely low MTR regions seen from DU-145 indicate possible tumor necrosis. Although there is no significant difference of MTR and redox ratio mean values (Figure 2), the mean CEST contrast for PC-3 (23.0±2.1%) is at a significant higher level ($p<0.05$) than DU-145 (1.9±6.7%) at +2 ppm, the peak of CEST asymmetric curve.

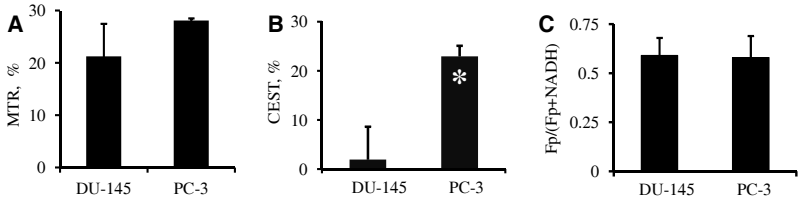

Figure 2. MTR and redox ratio are similar between tumor types. CEST contrast of PC-3 is significantly higher than DU-145 (*$p<0.05$).

Discussion and Conclusion: CEST and MT MRI are successfully employed to reveal the metabolic heterogeneity in prostate tumor xenografts and well correlated to redox scanning. These methods appeared to show consistently higher level of heterogeneity in DU-145 tumors than in PC-3 tumors at this particular stage of tumor progression. The distribution heterogeneity of the macromolecules and free amino acids appeared to correlate with the heterogeneity of mitochondrial redox state in tumors. Co-registered images of these prostate tumor xenografts by these three methods and at different progression time points will be further pursued.

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