CEST MRI as a Potential Imaging Biomarker of Mitochondrial Metabolic State of Breast Cancer

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Introduction: Tumor metabolism has become an active area of research recently. Abnormalities in mitochondrial metabolic state including redox state are found to be important factors in tumor progression. Previous work has shown mitochondrial redox state measured with redox scanning in human melanoma and breast cancer mouse xenografts can predict the tumor metastatic potential or aggressiveness [1,2]. We aim to investigate if non-invasive MRI methods could serve as surrogate metabolic/functional imaging biomarkers for tumor metastatic potential. The chemical exchange saturation transfer (CEST) effect from amide and hydroxyl protons have previously been exploited to measure pH [3] and liver glycogen [4]. CEST contrast using high magnitude of saturating RF pulse can be used to map fast exchanging free amino acids, such as brain glutamate (Glu) [5]. Glu also promotes cancer proliferation, migration and invasion of tumor cells [6]. As one of the major metabolites [8] in breast cancer exhibiting CEST effect, Glu is known to be connected with mitochondrial redox reactions through α-ketoglutarate in the TCA cycle. Therefore, we hypothesize that CEST effect from breast cancer amino acids (especially Glu) may be used as a non-invasive biomarker of mitochondrial metabolic state for predicting cancer metastatic potential.

Methods: Mice bearing Human breast cancer MDA-MB-231 (n=6) at different sizes (5-10 mm diameters) were scanned at a Varian 9.4-T horizontal MRI scanner using a 35-mm ^{1}H volume coil. CEST Z-spectra from -5 to 5 ppm were collected from tumor central cross-sections using a custom-programmed sequence, with a frequency selective rectangular 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 1-2 mm, flip angle 15^o, readout TR 6.3 ms, TE 3.0 ms, matrix size 128×128, and number of averages 2. One saturation pulse followed with 128 segment acquisition was repeated every 9 second. B_0 and B_1 maps were acquired for the correction of CEST contrast maps at +3ppm, normalized to -3ppm [5]. T_1 and T_2 weighted images of the same slice were also acquired with TR/TE= 100ms/3ms and 2s/160ms respectively. In a separate experiment, 50 μL Glu PBS solution (pH=6.5) were injected directly into a tumor. CEST images were collected at baseline, 45 and 90 minutes post Glu injection. After MRI, mice under anesthesia underwent snap-freezing procedures. Tumors were harvested and embedded for redox scanning [1,2]. All animal experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania.

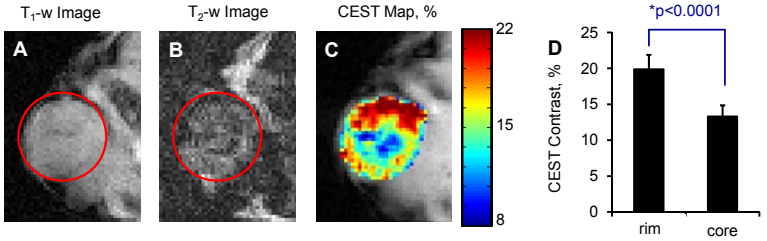

Figure 1. CEST map (C) shows clear core-rim heterogeneity of breast cancer contrasting the relatively uniform T_1 and T_2 weighted structural images (A and B). Tumor rim CEST contrast is significantly higher than the core (D). No tumor necrosis was observed based on T_1 and T_2 weighted images.

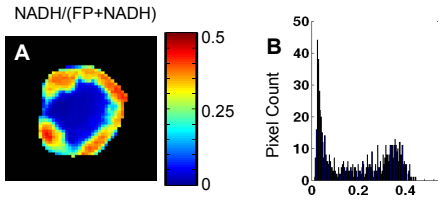

Figure 2. Typical mitochondrial metabolic image obtained by redox scanning reveals the similar pattern as the CEST map in Figure 1.C. Redox ratio histogram clearly displays the core-rim separation (B).

Results and Discussion: Clear core-rim heterogeneity of breast cancer is observed in the CEST map but not in T_1 and T_2 weighted structural images (Figure 1). CEST contrast at tumor rim is significantly higher than in the core (*p<0.0001), mimicking the pattern seen from mitochondrial redox scanning (Figure 2). Direct injection of Glu into a tumor increases tumor CEST contrast and gradually minimizes core-rim difference except at the injection site (Figure 3). Glutamate as an important factor for tumor growth and progression can be converted to α-ketoglutarate, a key component in the mitochondrial TCA cycle. Both conversion and the TCA cycle are mediated by NAD^+/NADH. Therefore, it is possible that Glu level may be coupled with mitochondrial redox state.

Conclusion: The results of this study showing a pattern correlation between CEST MRI contrast and mitochondrial redox state indicates that CEST MR could potentially be a new biomarker for mitochondrial metabolic state including redox state. While redox scanning is invasive, non-invasive CEST MRI has great potential of clinical translation.

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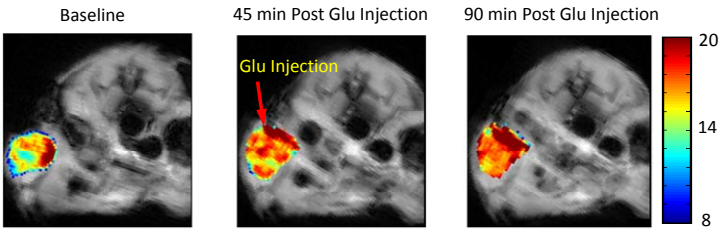

Figure 3. CEST maps at baseline, 45 and 90 min post direct injection of glutamate into a tumor. The glutamate injection increased tumor CEST effect and on the meantime gradually minimized core-rim difference except at the direct deposition site on the rim (arrow). Note: tumor orientation might be slightly changed due to injection and repositioning.

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