CEST-MRI detects metabolite levels for monitoring breast cancer cell aggressiveness

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Target audience: Investigators who are interested in cancer detection, especially in applications for detecting the aggressiveness of breast cancer.

Purpose: Altered metabolism is a hallmark of cancer, and aggressive tumors have a high metabolic activity. For example metabolites such as glutamine (Gln), glucose (Glc), lactate (Lac), phosphocholine (PC), and glycerophosphocholine (GPC) are biomarkers for cancer diagnosis. Tumor cells utilize their rewired metabolism to support their growth and proliferation. Thus *in vivo* assessment of these metabolite levels is important for cancer diagnosis, for development of anti-cancer treatments, and for treatment monitoring (1). Magnetic resonance spectroscopy (MRS) and magnetic resonance spectroscopic imaging (MRSI) are increasingly being used in the clinic in addition to conventional magnetic resonance imaging (MRI) for cancer diagnosis. However, MRS/MRSI is limited by its relatively low spatial resolution *in vivo*. With an increasing interest in monitoring metabolites for cancer diagnosis and treatment planning (2,3), there is a strong need for alternative methods to study these metabolite levels *in vivo*. In particular, metabolite levels that reveal the aggressiveness of tumors are of great interest. Chemical Exchange Saturation Transfer (CEST) is a molecular imaging approach that sensitively detects specific groups of exchangeable protons (4-6), such as hydroxyls in Glc (7-9) and amines in Gln and glutamate (Glu) (10,11). Here we have applied CEST to monitor exchangeable protons in metabolites in breast cancer cell lines with distinctive aggressiveness.

Methods: CEST imaging: All MR images were acquired at 310 K using a 500 MHz Bruker Avance system. CEST imaging was conducted through collection of two sets of saturation images, which were a water saturation shift referencing (WASSR) (13) set for B0 mapping and a CEST set for characterizing contrast. A modified rapid acquisition with relaxation enhancement (RARE) sequence including a saturation pulse was used (12). For the WASSR images (13), the saturation parameters were: t_{sat}=500 ms, B_1 =0.5 μT, TR=1.5 s with saturation offsets incremented from -1 to +1 ppm with respect to water in 0.1 ppm steps; for the CEST images they were: tsat=4 s, B1 =3.6 μT, TR=6 s, with offsets from -6 to +6 (0.2 ppm steps). The acquisition parameters were: TR=6.0 s, effective TE= 4.3 ms, RARE factor=16, slice thickness=1 mm. Sample preparation: Phantom solutions of metabolites were prepared in PBS at a concentration of 20 mM, including GPC, PC, threonine (Thr), choline (Cho), myo-inositol (mI), glycine (Gly), phosphocreatine (PCr), creatine (Cr), Glu, Gln, Glu+Gln (Glx), glutathione (GSH) and alanine (Ala). Cell extracts were freeze-dried after high-resolution (HR)-MRS, re-dissolved in PBS at pH 7.3. Three differentially aggressive human breast cell lines were used: nonmalignant MCF-12A breast epithelial cells, weakly malignant MCF-7 breast cancer cells, and highly malignant MDA-MB-231 breast cancer cells. Dual phase extraction was performed to separate the water-soluble and lipid-soluble phases, while macromolecules such as denatured proteins, DNA, and RNA were removed (14). The water-soluble phase contains small molecules such as amino acids, lactate, sugars, metabolites, and short peptides, among others. MRS: Fully relaxed, quantitative ^{1}H HR-MRS of the water-soluble phases was performed on the spectrometer and analyzed using the MestReC (ver4.9.9.6) as previously described (15). Notice that while water is located at 4.75 ppm in the normal proton spectrum, CEST spectra are referenced with respect to water at 0.0 ppm.

Results and Discussion: The CEST contrasts of some of the individual metabolites that were detected at relatively high concentrations by HR-MRS are shown in Fig. 1. In Fig. 2A, the HR-MRS study showed that levels of CEST-active metabolites were inversely correlated with the aggressiveness of breast cancer cells, in which the highly malignant MDA-MB-231 had the lowest levels of CEST-active metabolites among the three cell lines (i.e. MCF-12A>MCF-7>MDA-MB-231). CEST imaging of the same samples showed that the metabolite CEST contrast was the lowest in MDA-MD-231 (Fig. 2B), which corresponded to our simulation. The CEST contrast was highest at around 1 ppm away from water (Fig. 2C), which corresponds to mI, Glc, Glu, and Gln according to our measurements of individual metabolites (Fig. 1). Further simulations are underway to better understand the CEST effect observed in these breast cell extracts.

Conclusion: The CEST-MRI profiles of metabolites and amino acids were able to discriminate between nonmalignant human breast epithelial cells, weakly aggressive, and highly aggressive human breast cancer cells. By comparing the extracts of this panel of human breast cell lines using consecutive HR-MRS and CEST-MRI, we were able to semi-quantitatively assess the levels of metabolites by CEST-MRI, which was able to serve as a readout for the malignancy of cancers.

References: 1. Glunde K, et al. Semin Oncol 2011;38:26. 2. Haddadin IS, et al. NMR Biomed 2009;22:65. 3. Serkova NJ, et al. Bioanalysis 2012;4:321. 4. van Zijl PC, et al. Magn Reson Med 2011;65:927. 5. Ward KM, et al. J Magn Reson 2000;143:79. 6. Sherry AD, et al. Annu Rev Biomed Eng 2008;10:391. 7. van Zijl PC, et al. PNAS 2007;104:4359. 8. Chan KW, et al. Magn Reson Med 2012;68:1764. 9. Nasrallah FA, et al. J Cereb Blood Flow Metab 2013;33:1270. 10. Cai K, et al. Nat Med 2012;18:302. 11. Reynolds MR, et al. Oncogene 2013. 12. Liu G, et al. Contrast media & molecular imaging 2010;5:162. 13. Kim M, et al. Magn Reson Med 2009;61:1441. 14. Tyagi RK, et al. Magn Reson Med 1996;35:194. 15. Glunde K, et al. Cancer Res 2004;64:4270. **Supported by NIH R01 CA134695, R01 EB015031, R01 EB015032, P50CA103175**

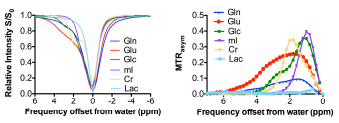

Fig. 1 Z-spectra and MTR_{asym} of metabolites, such as Gln, Glu, Glc, mI, Cr and Lac at 20 mM.

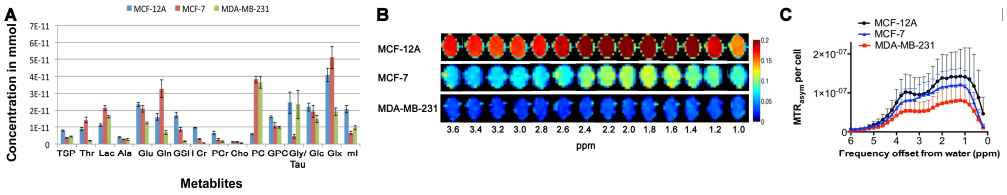

Fig. 2 HR-MRS and CEST of breast cell extracts. **A.** HR-MRS quantification of metabolites in MDA-MB-231, MCF-7 and MCF-12A cell extracts. **B.** CEST MTR_{asym} maps at 1-3.6 ppm for the breast cell extracts, **C.** CEST MTR_{asym} contrast of cell extracts normalized per cell (n=6).