AMINE AS A NOVEL BIOMARKER FOR DIFFERENTIATING MALIGNANCY OF BREAST CANCER CELLS

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Target audience: Investigators interested in the development of novel imaging biomarkers for clinical and pre-clinical applications in cancer.

Purpose: Assessing the malignancy and phenotype of cancer cells is vital for selecting optimal cancer treatments. Amide proton transfer (APT), a chemical exchange saturation transfer (CEST) technique, has been reported to show changes in brain tumors [1], but amine proton exchange from endogenous metabolites and free proteins/peptides side chains can also be a major contributor to CEST signals [2]. In the present work, we show how differences in amine proton exchange may be used to differentiate cancer cells with different malignant potential.

Methods:
Cell culture: The human breast cancer cell lines MCF-7 and MDA-MB231 were maintained in complete DMEM medium supplemented with 10% FBS. CEST measurements were performed on a 9.4 Tesla Varian/Agilent horizontal scanner. CEST data were acquired by continuous-wave saturation with 81 saturation offset frequencies ranging from −5 to +5 ppm relative to the water resonance, yielding high resolution (0.125 ppm) z-spectra. The z-spectra were normalized by the corresponding unsaturated signals and corrected for B_0 inhomogeneities. A two-shot, spin echo, echo-planar imaging (EPI) sequence was used with the following parameters: slice thickness = 2 mm, field of view = 10×10 mm^2, matrix size = 96×96. CEST data were analyzed using asymmetry analysis (MTR_{asym}) to remove the symmetric effects of direct water saturation.

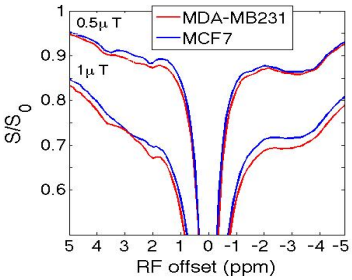

Figure 1: Comparison of MT Z-spectra of MCF7 (blue) and MDA_MB231 (red) cells at low saturation power (0.5 and 1μT).

Results: Off-resonance z-spectra at several saturation powers were obtained from low malignancy MCF7 cells and high malignancy MDA-MB231 cells. At low saturation power (0.5 μT or 1 μT), z-spectra of both MCF7 and MDA-MB231 cells showed a narrow saturation dip at + 3.5 ppm consistent with the APT effect (Figure 1) as well as a narrow dip at +2 ppm. However, at higher saturation powers ($B_1 \geq 2$ μT), the amide dip at + 3.5 ppm is not observed, but a broader dip that approximately spans the frequency range from + 2 ppm to + 3 ppm was observed (Figure 2) in MCF7 cells, which was not obvious in MDA-MB231 cells. This broader dip has previously been attributed to amine proton exchange [2]. As shown in Figure 3, there was a broad positive amine peak at high saturation power ($B_1 \geq 2$ μT) and the positive asymmetry is greater in MCF7 cells than in MDA-MB231 cells. The amine peak was much broader and more power dependent than the APT effect, consistent with a faster exchange rate. The correlation of changed amine signal with cell type suggests that amine CEST might serve as a new biomarker for differentiating types of cancer cells.

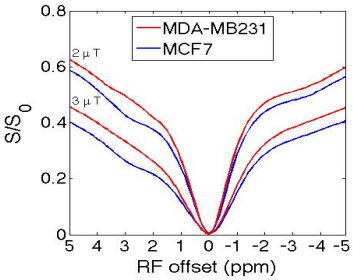

Figure 2: Comparison of MT Z-spectra of MCF7 (blue) and MDA_MB231 (red) cells at higher saturation powers (2 μT and 3 μT). Note a significant CEST dip at around 2-3 ppm from water in MCF7 cells, but not in MDA_MB231 cells.

Discussion: It has been proposed that APT contrast comes from mobile proteins and peptides [1]. Amine protons from cell metabolites, small molecules, and free protein/peptide side chains are also a source of endogenous CEST contrast [2,3 & 4]. Based on our data, when the saturation power is higher than 1.0 μT, amine exchange appears to be a major contributor to CEST from 2 to 5 ppm downfield from water. CEST contrast from amine groups depends on several variables, including concentration, exchange rate, chemical shift, as well as experimental parameters, but potentially amine proton exchange could be used to reflect endogenous mobile metabolite content. Amine proton exchange signals represent a weighted sum of many different amine groups, so further study is necessary to systematically determine the amine–proton exchange rates of the protein/peptides and different amino acids.

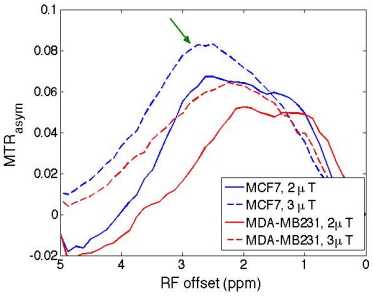

Figure 3: MTR_{asym} shows a large positive asymmetry, which is more prominent in MCF7 cells than MDA_MB231 cells. The amide peak at + 3.5 ppm is not observed, but amine proton peak centered in the 2–3 ppm range, can be seen at high powers.

Conclusion: Amine protons contribute to CEST signals under appropriate conditions and potentially may function as new endogenous biomarkers for non-invasively evaluating malignancy of breast cancer cells.

References: [1] Zhou et al. *Magn Reson Med*. 2003;50(6):1120-1126. [2] Jin *et al. Neuroimage.* 2012; 59(2):1218-1227. [3] Cai *et al. Nat. Med.* 2012;18: 302-306. [4] Ward *et al. J. Magn. Reson*. 2000;143(1): 79-87.