Chemical Exchange Saturation Transfer (CEST)-MRI detects free choline in breast cancer cells

Jannie P Wijnen*[1,2], Kannie WY Chan*[1,3], Peter CM van Zijl[1,4], Michael T McMahon**[1,4], and Kristine Glunde**[1]

[1]*Russell H. Morgan Department of Radiology and Radiological Sciences, Johns Hopkins University School of Medicine, Baltimore, MD, United States,* [2]*Department of Radiology, University Medical Centre Utrecht, Utrecht, Netherlands,* [3]*Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, Baltimore, MD, United States,* [4]*F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States*

***Authors contributed equal ** Authors share the corresponding authorship**

Introduction: Altered phospholipid metabolism is a hallmark of cancer. High levels of total choline-containing metabolites (tCho), phosphocholine (PC), and phosphoethanolamine (PE) have consistently been detected in malignant cancer types [1]. Relatively low levels of glycerophosphocholine (GPC) were found in breast [2] and ovarian cancer [3]. *In vivo* assessment of these metabolite levels is important for cancer diagnosis, for development of anti-cancer treatments [4], and for treatment monitoring. ^{31}P Magnetic Resonance Spectroscopy (MRS) can be used to study ^{31}P-containing metabolites involved in phospholipid metabolism, such as PC, GPC, PE, and glycerophosphoethanolamine (GPE). The tCho signal, which consists of the overlapping signals of free choline (Cho), PC, and GPC, can be detected with ^{1}H MRS. However; even high-resolution MRS (HR-MRS) cannot completely resolve these choline resonances due to overlapping signals of other compounds [4]. Hence, there is a strong need for alternative methods to study these metabolite levels *in vivo*. In particular the free choline levels have not been extensively investigated in cancer. Chemical Exchange Saturation Transfer (CEST) is a molecular imaging approach that allows identification of specific groups of exchangeable protons [5]. Here, for the first time, we applied CEST to monitor exchangeable protons on metabolites, such as Cho, PC, GPC, and PE.

Purpose: In this study, we have explored the use of CEST-MRI for detecting phospholipid metabolites in breast cancer cells.

Methods: CEST imaging: All MR images were acquired at 310 K using an 11.7 T Bruker Avance system. CEST imaging was conducted through collection of two sets of saturation images, which were a water saturation shift referencing (WASSR) [6] 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 to acquire saturation images [7]. For the WASSR images, the saturation parameters were: tsat=500 ms, B1 =0.5 μT, TR=1.5 sec with saturation offsets incremented from -1 to +1 ppm with respect to water in 0.1 ppm steps, while for the CEST images they were: tsat=4 sec, B1 =3.6 μT, TR=6 sec, with offsets from -6 to +6.0 (0.2 ppm steps). The acquisition parameters were: TR=6.0 sec, effective TE= 4.3 ms, RARE factor=16, slice thickness=1 mm. Sample preparation: Phantom solutions of Cho, ethanolamine (Etn), PC, GPC, and PE were prepared in phosphate buffered saline (PBS) at a concentration of 5 mg/ml. Cell extracts were freeze-dried after HR-MRS, re-dissolved in PBS, and their pH was adjusted to 7.4. Three differentially aggressive human breast epithelial and breast cancer cell lines – nonmalignant MCF-12A breast epithelial cells, weakly malignant MCF-7 breast cancer cells, and highly malignant MDA-MB-231 breast cancer cells – were grown to 70% confluence. 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 [8]. The water-soluble phase contains small molecules such as amino acids, lactate, sugars, choline- and ethanolamine-containing metabolites, and short peptides, among others. MRS: Fully relaxed, quantitative ^{1}H HR-MRS of the water-soluble phases was performed on a Bruker Avance 500 MHz MR spectrometer and analyzed using the MestReC 4.9.9.6 software as previously described [9]. 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: CEST contrast of metabolites involved in phospholipid metabolism clearly showed that Cho had the highest contrast at an offset frequency of 1.2 ppm (Fig. 1a) from water. Etn displayed CEST contrast as well, but its peak was much broader than that of Cho. The phosphorylated metabolites PC, GPC, and PE did not show detectable CEST contrast. Since Cho displayed the strongest CEST contrast, we explored its sensitivity (Fig. 1b,c). The CEST approach had a good sensitivity *in vitro* at a choline concentration of 25 mM (Fig. 1b). Furthermore, the water line was narrower at lower Cho concentrations, which is favourable for *ex vivo* and *in vivo* detection of biological specimens because the Cho levels will be lower in these situations. Based on this, we decided to look at tumor cell extracts. These showed a clear CEST contrast at a frequency of 1.2 ppm (Fig. 2a), which is characteristic of Cho. The Cho level was the highest in MCF-12A cells, and the lowest in MDA-MB-231 cells (Fig. 2c), which is in agreement with previous studies of HR-MRS of these cell lines [9]. The cellular Cho concentration inversely correlates with the degree of malignancy of the investigated breast epithelial and cancer cell lines. The CEST contrast at 1.2 ppm in the cell extracts (Fig. 2b) correlates well with the Cho levels measured by ^{1}H HR-MRS, which means that the CEST signal intensity has potential to be used as a semi-quantitative measure for Cho and possibly other metabolite levels. This non-invasive approach of imaging the Cho level as an inverse biomarker of malignancy can potentially be useful in future clinical research studies because it allows for detecting an otherwise inaccessible metabolite, which is influenced by altered Cho transport and choline kinase alpha expression and activity in cancers [4].

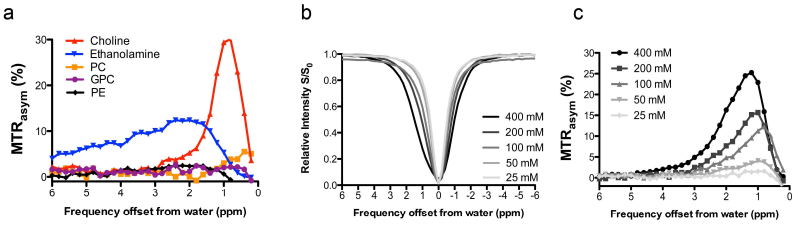

Figure 1. a) CEST contrast (MTR_{asym}) of metabolites involved in the phospholipid metabolism, such as choline, ethanolamine, phosphocholine (PC), glycerophosphocholine (GPC), and phosphoethanolamine (PE); b) z-spectra of choline solutions at 25, 50, 100, 200 and 400 mM and c) their MTR_{asym}.

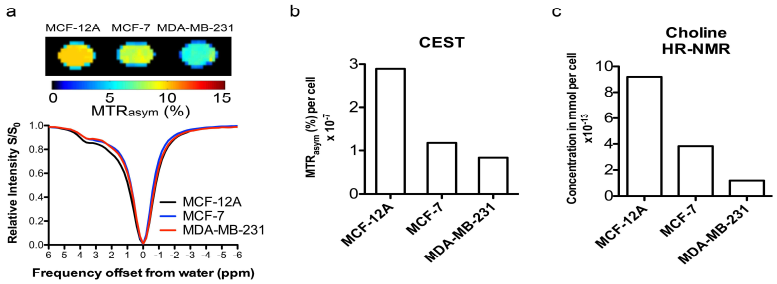

Figure 2. Metabolite levels in cell extract. a) CEST contrast (MTR_{asym}) map of water soluble metabolites extracted from MCF-12A, MCF-7 and MDA-MB-231 cells at 1.2 ppm (top panel) and Z-spectra(bottom panel); b) CEST contrast at 1.2 ppm of these cells normalized to the number of cells; c) Choline concentration in cell extracts measured by ^{1}H HR-MRS.

Conclusion: Our data suggest that CEST-MRI allows for detecting Cho levels in cell extracts, which cannot easily be detected by any other MRS method. The CEST enhanced signal intensity is 1-3% of water, corresponding to 1-3M sensitivity. This opens up the possibility of imaging Cho levels *in vivo*, providing information about its concentration and its spatial distribution. **Future directions:** We are investigating the possibility of using this approach for detecting other metabolites related to phospholipid metabolism, which could provide a novel molecular imaging approach for investigating breast cancers *in vivo*.

References: *[1] Podo F, NMR Biomed. 1999;12(7) [2] Aboagye EO et al., Cancer Res. 1999 Jan 1;59(1) [3] Iorio E et al., Cancer Res. 2010;70(5) [4] Glunde K et al., Semin. Oncol. 2011,Feb;38(1) [5] van Zijl PCM et al., Magn. Reson. Med. 2011;65(4). [6] Kim M et al., Magn. Reson. Med. 2009;61(6) [7] Liu et al., Contrast Media Mol. Imaging, 2010;5(3) [8] Tyagi et al. MRM 1996 Feb;35(2) [9] Glunde et al., Cancer Res 2004 Jun 15;64(12).*

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