ASSESSMENT OF MEMBRANE FLUIDITY USING NUCLEAR OVERHAUSER ENHANCEMENT MEDIATED MAGNETIZATION TRANSFER

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Target audience: Investigators developing or applying new MRI methods for characterization of tumors.

Purpose: Membrane fluidity plays a key role in vital cellular functions. We have observed a novel source of contrast at around -1.6 ppm in z-spectra which we postulate is derived from nuclear Overhauser enhancement (NOE) effects between water protons and choline-containing metabolites which are in a restricted state within cell membranes [1,2]. Alterations in membrane fluidity may dramatically affect the MR visibility and intermolecular interactions of such restricted metabolites. In this work, we examine whether changes in the NOE at -1.6 ppm within tumors can be measured *in vivo* using NOE-mediated MT contrast, and show that such changes may arise from altered membrane fluidity in different mammalian cell lines.

Methods: Cell lines: HEK293 cells originally derived from human embryonic kidney were obtained from ATCC. SH-SY5Y cells, a neuroblastoma cell line which can be differentiated to a more mature neuron-like phenotype, were also purchased ATCC. Both HEK293 and SH-SY5Y cells were cultured in Dulbecco's Modified Eagles Medium supplemented with 10% heat inactivated fetal bovine serum (FBS) in a 5% CO2 humidified incubator. 10 mM retinoic acid (RA) was used as sole differentiation factor.
Animal model: Neuroblastomas were produced inside the brains of rats by intracortical injections of 1×10^5 cells.
MT measurements: Continuous-wave (CW) MT sequences, which contain an 8 s rectangular irradiation pulse followed by readout, were performed on a 9.4 Tesla Varian horizontal scanner. Irradiation power was optimized to 1 μT on both rat brain and cells. The saturating pulse offset was varied from -5 to +5 in 81 steps to obtain a z-spectrum. Signal changes downfield from water corresponding to NOE effects were measured from these spectra for both tumor and contralateral normal tissue.
Membrane fluidity measurement: cellular membrane fluidity was measured by a fluorescence method which is based on the lateral diffusion rate of fluorescent aromatic hydrocarbon pyrene [3].
Results: Figure 1(left) shows significant NOE-mediated MT signals around -1.6 ppm in normal tissue but not in tumor. Figure 2(middle) shows the z-spectra for SH-SY5Y cells before and after differentiation. A clear dip at around -1.6 ppm was found on the SH-SY5Y cells after differentiation, but not before. Fluorescence measurements show that the membrane of SH-SY5Y cells before differentiation is more fluid than after differentiation (Figure 1 right). As shown in Figure 2 (left), compared to control HEK293 cells, z-spectra for HEK293 cells cultured with cholesterol supplement showed an increased -1.6 ppm dip; however the -1.6 ppm dip decreased with cholesterol depletion from the membrane. Fluorescence measurements confirmed that the membrane fluidity changed when cells were cultured under different culture conditions (Figure 2 middle). There is a good correlation (R^2=0.9858) between the change of membrane fluidity and NOE-mediated MR signal change (Figure 2 right).
Discussion: Our results indicate that the NOE-mediated MT signal may depend on membrane fluidity *in vitro* and potentially therefore be used to evaluate pathological conditions *in vivo*. Here, we cultured cells with cholesterol supplement or depletion. Cholesterol has been reported works as one of the key regulators for membrane fluidity because cholesterol is able to bind to phospholipid, resulting in more rigid membrane fluidity [4]. From earlier *in vitro* studies, the visibility and magnitude of NOE effects in choline compounds in plasma membranes depends strongly on their degree of restriction (immobilization). The changes observed in different cell lines likely reflect the mobility status of choline head groups in the membrane.
Conclusion: Membrane fluidity is an important membrane characteristic that can not be measured *in vivo*. This work shows that NOE-mediated MT may provide a non-invasive and novel imaging method to assess alterations in membrane fluidity.
References: [1] Zu *et al. Magn Reson Med.* 2014;72: 471-476. [2] Xu *et al. NMR Biomed.* 2014;27: 406-416. [3] Parmahamsa *et al. Alcohol Alcohol*. 2004 ; 39 :110-112. [4] Cooper RA. *J Supramol Struct*. 1978;8: 413-430.

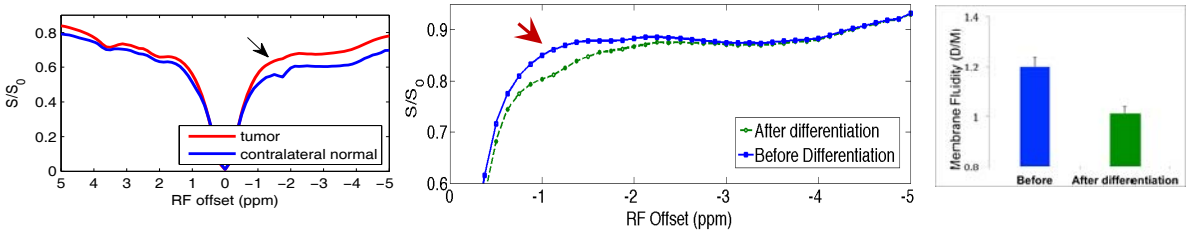

Figure 1: ***Left:*** *MT Z-spectra on tumor (red) and contralateral normal tissue (blue) in a tumor-bearing rat. Note the significant NOE-mediated MT signal at around -1.6 ppm from water in normal tissue, but not in tumor.* ***Middle:*** *Z-spectra on SH-SY5Y cells before (blue) and after (green) differentiation. Note the obvious -1.6 ppm dip after differentiation.* ***Right:*** *Membrane fluidity of SH-SY5Y cells before (blue) and after differentiation (green). Note higher value of M/D ratio represents higher membrane fluidity.*

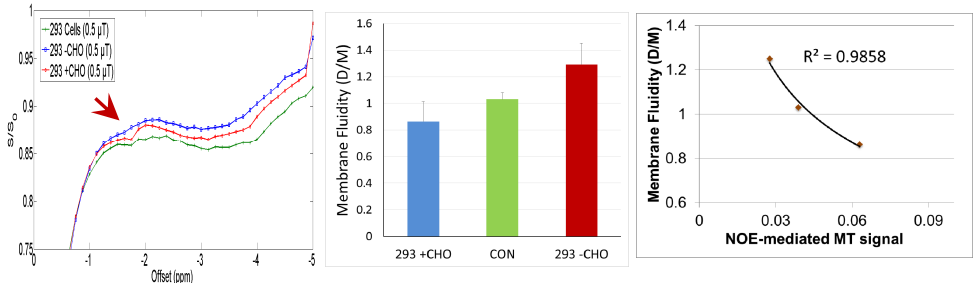

Figure 2: ***Left:*** *Z-spectra on HEK293 cells cultured with normal medium (green), cholesterol supplement (red), or cholesterol depletion (blue).* ***Middle:*** *fluorescence measurement of membrane fluidity when HEK293 cells cultured at different conditions.* ***Right:*** *The correlation between membrane fluidity and NOE-mediated MR signal.*