A MRI-CEST Method for mapping Water Cycling across Cellular Membranes

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INTRODUCTION:
Water cycling across the membrane transporters is a hallmark of cellular metabolism and it could be of high diagnostic relevance in the characterization of tumors and other diseases. Herein, we report a CEST-MRI method that allows to gain insights into the water exchange across compartments by exploiting the response of the intracellular $CEST_{@2ppm}$ (1) signal to the presence of extracellular Gd-based contrast agents (Fig.1A)**.**

METHODS:
The paramagnetic effect of Gd-HPDO3A has been firstly assessed *in vitro* on the CEST response of endogenous and exogenous molecules entrapped in liposomes. Next, the paramagnetic effect on the endogenous CEST signal was acquired on RBCs and on breast cancer (BCs) cells endowed with a different degree of malignancy, *i.e*. 4T1 (*high*), TS/A (*medium*) and 168FARN (*low*), either as cells' pellets or as transplantable murine models.

RESULTS:
By using liposomes of different size and water permeability (modulating formulation with saturated/unsaturated phospholipids) the drop of CEST response in the presence of Gd-HPDO3A can be accounted essentially in terms of the different membrane permeability to water molecules. By applying the approach to cellular systems, the following order of permeability 4T1>TS/A>168FARN>RBC was obtained. *In vivo* results on tumors are reported in Fig.1B-D, and kinetic curves of the averaged $ST_{@2ppm}$ *vs*. time after the *i.v.* injection of Gd-HPDO3A in Fig.1E-G, respectively. Fig.1H reports the histograms upon clustering the voxels based on water permeability.

DISCUSSION:
The results show how the CEST response from molecules entrapped in a compartment can be made strongly dependent on the exchange with the water molecules of the outer compartment loaded with a paramagnetic agent. The determinant of this effect is the value of the relaxation rate of water protons in the inner compartment. The R_1 change in the inner compartment directly reports on the extent of water cycling across the two compartments. When the concept is applied to cellular systems, the different ST% drop reports on the differences in the transport proteins on the cell's membrane, in turn is related to cell metabolism and tumor aggressiveness.

CONCLUSION:
Whereas these data were consistent with previous relaxometric studies *in vitro*, the method appears highly innovative as it allows to measure water cycling *in vivo* by generating maps reporting, *voxel by voxel*, the permeability distribution in the tumor region (2).

REFERENCES:
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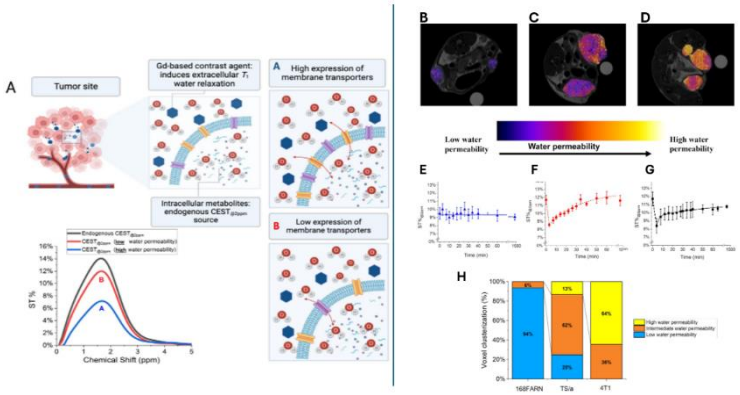

Figure 1. (A) Chart showing the rationale of the *in vivo* experiments. (B-C-D) Water permeability MRI maps for 168FARN, TS/A and 4T1 murine models, respectively. (E-F-G) Kinetic curves of the averaged $ST_{@2ppm}$ *vs*. time after the i.v. injection of Gd-HPDO3A bolus (H)Histogram reporting clusterization of voxels.