

Generation of Multicolor/multiparametric MRI-CEST maps of murine tumor by using YbHPDO3A and Dy-loaded Red Blood Cells.

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Purpose: Three key hallmarks of the tumor development are represented by the increased vascular volume, the enhanced vascular permeability and the acidification of the extracellular/ extravascular pH (pHe), respectively. The possibility to visualize simultaneously the three biomarkers by MRI may be highly relevant to assess tumor staging and to evaluate early response to therapeutic treatments. CEST contrast agents appear well suitable to tackle this task by using the currently available ¹H-MRI scanners. YbHPDO3A, a small hydrophilic, well tolerated paraCEST agent, chemical analogue of the clinically approved ProHance, provides the way to get information both on change in pHe and on change in vascular permeability. To obtain quantitative information on the vascular volume, Dysprosium- loaded Red Blood Cells, that act as highly sensitive CEST-MRI agents have been used. The outstanding sensitivity of Dy-loaded RBCs (less than 1pM in term of cell concentration, the highest displayed for CEST agents), together with their good biocompatibility, makes them excellent companion of the molecular YbHPDO3A probe for an improved tumor characterization. **Methods:** Six to ten weeks female Balb/C mice (Charles River Laboratories, Italy) were subcutaneously inoculated with 5×10^5 TSA murine breast cancer cells and tumors were analyzed 10 days from the injection (Fig.1A, representative T_{2w} image of mouse bearing subcutaneous tumor). Murine Red Blood Cells were isolated from the blood and loaded with DyHPDO3A by applying the hypotonic swelling procedure (loading step: 30min, 100mM DyHPDO3A, overall osmolarity of 160mOsm/l; membrane resealing step: 20min, overall osmolarity of 280mOsm/l). The intracellular metal content was evaluated by ICP-MS measurement on cell lysates. The MRI experiment consists of : i) acquisition of pre-injection images, ii) acquisition of images post Dy-loaded RBCs *i.v.* injection and iii) acquisition of images post *i.v.* injection of 1.2mmol/kg of YbHPDO3A. The images were recorded on a 7T on a Bruker Avance 300 Spectrometer equipped with microimaging probe. Z-spectra were acquired by using a RARE spin echo sequence (TE=3ms, TR=5s) with isotropic 63x64 acquisition matrix, FOV of 3 cm and slice thickness of 1mm. The whole sequence was preceded by a saturation scheme consisting in a continuous rectangular wave pulse 2s long with a RF pulse of 3 μ T for Dy-loaded-RBCs and 24 μ T for YbHPDO3A, respectively. **Results:** RBCs loaded with DyHPDO3A show a very intense CEST peak (*ca.*75%) close to 4ppm from water signal (Fig.1B). Upon *i.v.* injection of Dy-loaded-RBCs the Z-spectrum in the tumor region clearly shows the Dy-RBCs ST signal (*ca.* 5% at 4 ppm, Fig. 1C). The ST_{map} at 4ppm reported in Fig. 1D indicates large heterogeneity in the ST% characterizing the tumor voxels that has been associated to an unbalanced distribution of blood vessels in the tumor . Then, upon *i.v.* injection of YbHPDO3A, the ST map at 67ppm, *i.e.* at the specific absorption of one of the two YbHPDO3A isomers, has been used as marker of the tumor perfusion (Fig.1E). Upon comparing the two maps, one get insight on the relationship between microvascularity and vessel permeability. Finally, as reported in Fig.1F, by applying the ratiometric approach the map of pHe has been obtained. **Conclusion** Dy-loaded RBCs represent a very innovative and highly sensitive new-entry in the family of CEST MRI contrast agents that in association with the YbHPDO3A probe may improve the tumor characterization. In fact, the vascular volume, the vascular permeability and the pHe are well known to be correlated to the tumor staging. From the multicolor/multiparametric maps obtained by applying CEST MRI,semi-quantitative information about these three tumor hallmarks may be extracted. **References** Delli Castelli D., Ferrauto G. Cutrin J.C., Terreno E., Aime S. Magn Reson Med. 2013 Mar 25. doi: 10.1002/mrm.24664.

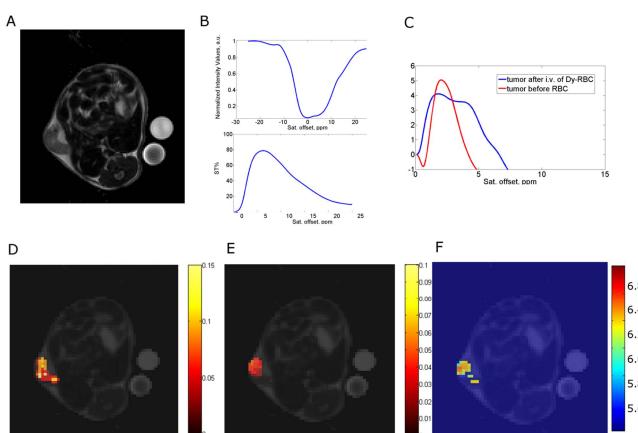


Figure 1. (A) T_{2w} image of mouse bearing tumor; (B) Z- and ST- spectra of Dy-loaded RBCs in the reference tube; (C) ST-spectra of tumor region pre and post Dy-RBCs injection; (D) ST map at 4ppm for vascular volume map; (E) ST map at 67 ppm for vascular permeability map; (F) Ratiometric map for pH measurement.