

Molecular Imaging of Cancer with Paramagnetic Vesicles Targeted to Phosphatidylserine

Patrick Winter¹, John Pearce¹, Zhengtao Chu², Jing-Huei Lee³, and Xiaoyang Qi²

¹Radiology, Cincinnati Children's Hospital, Cincinnati, OH, United States, ²Hematology and Oncology, University of Cincinnati, Cincinnati, OH, United States,

³Center for Imaging Research, University of Cincinnati, Cincinnati, OH, United States

Target Audience: Basic researchers interested in targeted MRI contrast agents for mapping the expression of cancer biomarkers.

Purpose: Neuroblastoma represents 15% of cancer deaths in children despite aggressive treatment with surgery, radiation therapy and chemotherapy. Accordingly, there is a pressing clinical need for improved diagnostic tools for combating neuroblastoma. Molecular imaging approaches offer improved sensitivity and specificity, which are crucial for early diagnosis and monitoring therapeutic effect. An MRI contrast agent specifically targeted to cancer biomarkers could be translated into clinical medicine to improve the management of patients suffering from neuroblastomas or other devastating cancers.

SapC (SapC) is a multifunctional protein that preferentially binds to unsaturated, negatively charged phospholipids, such as phosphatidylserine. Phosphatidylserine is expressed by tumor cells and their supporting vasculature, but not normal tissues. SapC can be incorporated onto the surface of phospholipid vesicles for selective targeting of tumors, such as neuroblastoma.

Methods: A lipophilic gadolinium chelate, Gadolinium-DTPA-bis(stearylamide) (Gd-DTPA-BSA), was incorporated onto the surface of SapC vesicles forming paramagnetic vesicles with an average diameter of 200 nm. Vesicles containing 55% Gd-DTPA-BSA (molar ratio of phospholipids) were imaged at 7T to measure the relaxivity compared to a standard MRI contrast agent, Gd-DTPA. The longitudinal relaxation rates (R1) of serial dilutions of paramagnetic SapC vesicles and Gd-DTPA were plotted against the gadolinium concentration (for Gd-DTPA) or the vesicle concentration (for Gd-DTPA-BSA/SapC vesicles) in each sample to determine the relaxivity.

The in vitro targeting ability of our MRI contrast agent was studied by adding paramagnetic SapC vesicles to the media of cultured human neuroblastoma cells (CHLA-20). The cells were washed, removed from the culture dish and centrifuged into pellets. Cell pellets were imaged at 7T to generate relaxation time (T1) maps. The in vivo targeting ability of Gd-DTPA-BSA/SapC vesicles was tested in a mouse with a subcutaneous CHLA-20 tumor. MRI was performed at 7T to map the R1 values of tumor and muscle before, immediately after and 22 hours after intravenous injection of 290 μ L of vesicles containing 1.34 mM Gd-DTPA-BSA.

Results: At 7T, Gd-DTPA had a relaxivity of $4.84 \text{ (s}^* \text{mM)}^{-1}$ relative to the gadolinium concentration. Gd-DTPA-BSA/SapC vesicles had approximately 2000 gadolinium chelates per particle, yielding a relaxivity of $2820 \text{ (s}^* \text{mM)}^{-1}$ relative to the vesicle concentration.

Gd-DTPA-BSA/SapC vesicles successfully targeted cultured human neuroblastoma cells (FIGURE 1). The T1 of untreated cells was 2607 ± 30 ms. Cells treated with a low concentration of vesicles (17 μ M Gd-DTPA-BSA) had a T1 of 2109 ± 90 ms, while a higher vesicle concentration (70 μ M Gd-DTPA-BSA) reduced the T1 further to 1167 ± 56 ms. Cells treated with a much higher concentration (1.12 mM Gd-DTPA-BSA) of untargeted vesicles (no SapC) had a T1 of 2042 ± 26 ms. In vivo imaging with paramagnetic SapC vesicles showed preferential uptake of the contrast agent in the tumor (FIGURE 2). The tumor relaxation rate (R1) was normalized to the R1 of skeletal muscle. Before injection, the R1 averaged over the entire tumor was 0.375 (s)^{-1} , while the muscle R1 was 0.536 (s)^{-1} , yielding a normalized tumor value of 0.700. Immediately after injection, the normalized tumor value increased to 0.760, an increase of 9%. Twenty-two hours after injection, the tumor increased further to 0.821, an increase of 17% over the pre-injection value.

T1 Mapping of Gd-SapC Vesicle Treated CHLA-20 Cells

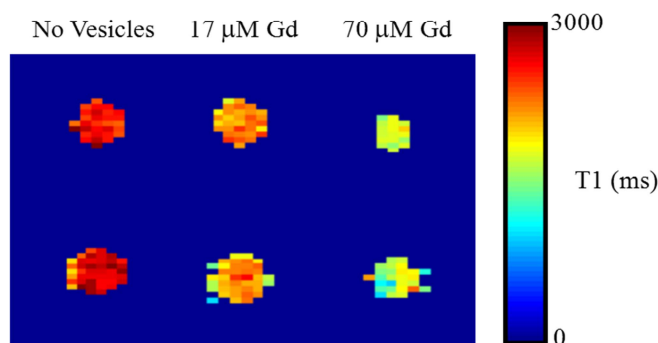


FIGURE 1: MRI (7T) of cells treated with Gd-DTPA-BSA/SapC vesicles. The T1 of untreated cells was 2607 ms. Treatment with 17 μ M Gd-DTPA-BSA lowered the T1 to 2109 ms, while 70 μ M Gd-DTPA-BSA reduced the T1 to 1167 ms.

R1 mapping (7T) of Gd-SapC Vesicle Treated Mouse Tumor

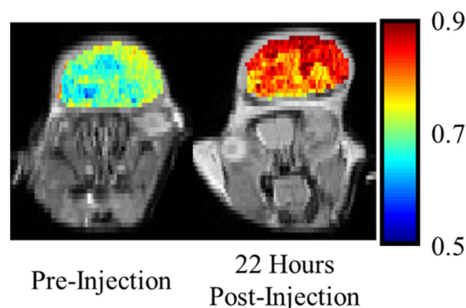


FIGURE 2: MRI (7T) of a tumor bearing mouse before and after injection of Gd-DTPA-BSA/SapC vesicles. Before injection, tumor R1 (normalized by the muscle R1) was 0.700. Twenty-two hours after injection, the normalized tumor R1 increased by 17%, to 0.821.

Discussion: Molecular imaging agents that are targeted to specific cancer biomarkers could provide earlier diagnosis and noninvasive characterization of tumors. A tumor targeted MRI contrast agent would be invaluable for diagnosing, staging and monitoring therapeutic response in children inflicted with neuroblastomas or other cancers. These experiments demonstrate that paramagnetic SapC vesicles can target tumors in vivo, providing noninvasive mapping of the cancer biomarker phosphatidylserine.