Specific MRI Detection of PSMA-expressing Prostate Cancer uisng 3C6 MAb-conjugated SPIONs

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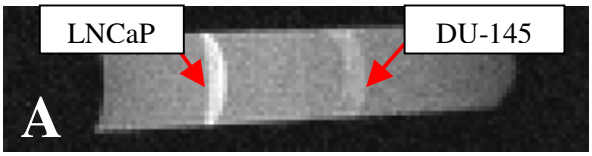
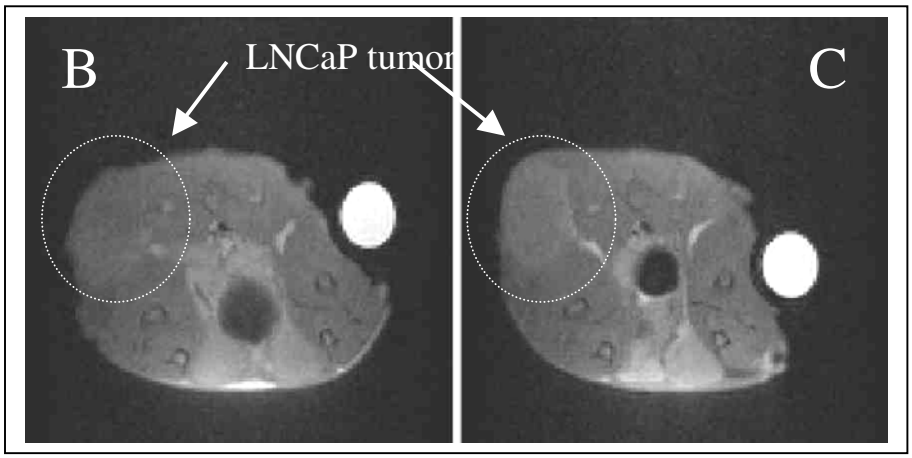
Introduction: New detection methods are needed for prostate cancer, particularly for metastatic disease, in order to provide patients the best possible staging and treatment. Our aim is to locate primary tumors or distant metastases using MRI with contrast agents targeted specifically to cancer cells. We take advantage of the fact that many types of prostate cancer cells express high levels of prostate specific membrane antigen (PSMA) on their cell surface. The imaging strategy is to use superparamagnetic iron oxide nanoparticles (SPIONs), attached to an antibody that binds to the extracellular domain of PSMA, to specifically enhance the contrast of PSMA-expressing prostate cancer cells. The use of antibody-conjugated MRI contrast agents to specifically target cancer cells has been demonstrated previously for some other cancers.[1] The use of monoclonal antibody (Mab) J591, conjugated to a radioisotope, has been used for gamma camera imaging of PSMA-expressing cancers.[2] Here, we demonstrate an MRI contrast agent targeted specifically to the external cell surface of PSMA-expressing prostate cancer cells.

Methods: A number of cell lines were investigated by flow cytometry and reverse transcriptase polymerase chain reaction to determine their level of expression of PSMA. Two cell types were chosen for MRI experiments: LNCaP cells, which express a high level of PSMA, and DU-145 cells, which do not express PSMA. Conjugation of Mab 3C6 to commercial streptavidin SPIONs (MACS® beads, 50 nm diameter, 57% iron oxide by weight) was achieved using the standard avidin-biotin reaction. LNCaP and DU-145 cells were cultured *in vitro*, allowed to bind with biotinylated 3C6 antibodies, and then incubated with streptavidin SPIONs. The cells and bound contrast agent were separated from unbound SPIONs by repeated centrifugation. The cells were then resuspended in agarose gel and layered into a plastic test tube. In vitro imaging was performed in a 1.9 T Oxford horizontal bore magnet using a Tecmag Libra spectrometer, Resonance Research shielded gradient set, and a Morris Instruments birdcage probe. The T_1 and T_2 contrast were investigated using 2D spin echo (T_1-weighted ,T_2-weighted), inversion-recovery-prepared 1D spin echo, and 2D gradient echo imaging sequences. For the *in vivo* experiments, the streptavidin SPIONs were labeled with biotinylated 3C6 antibodies and administered by tail vein injection (10 mg Fe/kg) to a 25-g male nude mouse with a roughly 1 cm diameter subcutaneous LNCaP tumor on its left flank. Multi-slice T_1-w spin echo imaging of the mouse (TE = 9 ms, TR=0.5 s, 0.25 mm resolution, 32 mm FOV, 2.5 mm slice thickness) was performed using a 1 T MRTechnology permanent magnet MRI system and halothane anesthesia.





Results and Discussion: Fig. A shows a T_1-w 2D spin echo image (TE = 5 ms, TR = 0.5 s) of the *in vitro* sample. The small radius of the MACS® beads results in rapid spatial variations of the magnetic field in the regions immediately surrounding the beads. The diffusion of water through these more rapidly varying fields leads to a short correlation time for magnetic field fluctuations which causes significantly enhanced T_1 relaxation. The LNCaP cells show good T_1 contrast, while the DU-145 cells show weak contrast relative to the background agarose. MACS® beads cause comparatively weak T_2 relaxation effects. In the *in vivo* image acquired ~30 minutes after injection of the contrast agent (Fig. B), the tumor intensity is similar to that of muscle. At 23 hours post-injection (Fig. C), the tumor appears bright compared to the surrounding muscle, suggesting that binding of the contrast agent to the tumor cells has occurred.

Conclusions: We have demonstrated *in vitro* that one can synthesize and image an MRI contrast agent that specifically binds to PSMA-expressing prostate cancer cells, using Mab 3C6 conjugated to commercial SPIONs. Preliminary experiments in mice with human prostate cancer suggest that this new imaging agent also leads to specific enhancement of T_1 contrast *in vivo*.

References: [1] e.g., Remsen LG, et al. Am J. Neuroradiol. 1996;17:411-8; Artemov D, et al. Magn. Reson. Med. 2003;49:403-8. [2] Bander NH, et al. J. Urol. 2003;170:1717-21.