Epidermal Growth Factor Receptor (EGFR) Targeted MRI Using A Probe of SPIO Nanocrystal and scFv EGFR Conjugate

H. Mao[1], L. Yang[2], X. Peng[2], Y. A. Wang[3], X. Wang[4], Z. Cao[2], G. Adams[5], Q. A. Yuan[5]

[1]Department of Radiology and Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia, United States, [2]Department of Surgery and Winship Cancer Institute, Emory University School of Medicine, Atlanta, Georgia, United States, [3]Ocean NanoTech, LLC, Fayetteville, Arkasas, United States, [4]Department of Radiology, Emory University School of Medicine, Atlanta, Georgia, United States, [5]Fox Chase Cancer Center, Philadelphia, Pennsylvania, United States

Introduction An important approach in developing target specific MRI probe for molecular imaging is to design and assemble functional biomolecules such as an antibody or receptor to a strong MRI contrast agent via conjugation. The complex of such imaging probe should have a proper half time in vivo and can sustain physiological conditions, for example the enzymatic degradation, in order to specifically interact with the imaging target. Here we report our investigation of a novel MRI probe consisting of superparamagnetic iron oxide (SPIO) nanocrystals conjugated with the protein (single chain antibody, scFv EGFR), developed for MR imaging of the epidermal growth factor receptor (EGFR) that is over expressed due to the tumor proliferation and growth.

Experimental Methods

Preparation of SPIO Nanocrystals and Coating SPIO nanocrystal colloidal was prepared based on previously reported method [1] with modifications. The core size of the nanocrystals is highly uniformed and can be controlled at 4, 9, 16 nm. Resulting SPIO nanocrystals then were coated with amphiphilic polymers similar to that developed by Gao et al. [2]. The hydrocarbon chains of the polymer intercalate into the inner hydrophobic layer that stabilize SPIO nanocrystal surface while carboxylic acid groups in the outer layer make the IO nanocrystal hydrophilic and reactive for conjugating a protein or peptide (shown in the scheme).

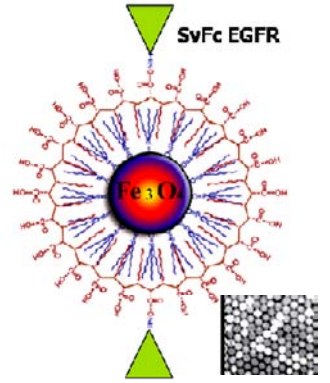
Conjugating Functional peptide The His-tagged human EGFR single chain antibody (scFv EGFR) was produced in a bacteria-expression system and conjugated to the amphiphilic polymer coated SPIO nanocrystals via carboxyl groups and a Ni-NTA chelating compound [2]. IO nanocrystal preparation with a core size of 9 nm was used in this study.

Specific Binding of SPIO Conjugates to EGFR+ Cells in vitro To test the binding specificity of SPIO-scFv EGFR, we used MRI T2 measurement and EGFR+ breast cancer cell lines including MDA MB-231 in vitro. Various formations of SPIO nanocrystals, e.g., wide type SPIO, complexes of SPIO-scFv EGFR and IO-GFP were incubated with MDA MB-231 cells (~$2x10^6$) respectively at 37°C for 1 hr before being collected and washed to rid of non-bound reagents. In addition to analyses using routine binding assay, cells were resuspended in the 1% agoras gel homogenously and then were arranged in the panels of a 1-mL multi-well plate for MRI study.

MRI All MRI experiments were performed on a 3T scanner (Philips Intera). For imaging SPIO nanocrstals solution and cell phantoms, a 10-cm circular surface was used. T1 or T2 weighted images and T1 or T2 measurements were obtained from each phantom using the method reported before [1]. In vivo imaging was carried out in mice with a 5-cm wrist coil. Typical imaging parameters for pre- and post-contrast spin echo imaging include: 20 coronal slices with slice thickness of 1.1 mm (no gap), TR/TE=450/13ms, rectangular FOV of 4 cm (2 cm in RL), matrix of 190^2 and NSA of 4. Animal was anesthetized using the mixture of ketamine/xylazine (90/10mg/kg) i.p. The SPIO nanocrystal agents (100μl) were administrated intravenously through the tail vein.

Results and Discussions Amphiphilic polymer coated SPIO nanocrstals demonstrated a wide range of MRI contrast effects in T1, T2 and T2*as a function of SPIO nanocrystal concentrations with the strong T1 contrast at the lower concentration and predominately T2 and T2* contrast when the SPIO concentration increased. The experiment of T2 measurements of SPIO nanocrystals treated cell cultures in vitro demonstrated that the specific binding of SPIO-scFv EGFR conjugate to the EGFR over-expressed MDA-MB-231 breast cancer cells (Figure 1) with the significant reduction of T2 when compared to the wide type SPIO and the SPIO-GFP complex, which does not have specific binding to EGFR. In vivo imaging showed a rapid uptake of SPIO nanocyrstals in the live and spleen. Furthermore, we observed the signal enhancement in the kidney cortex about 30 min. after i.v. introducing SPIO-peptide complex. The cortex signal was further enhanced to the maximum over the next hours (Figure 2). This T1 enhancement in the renal cortex remained until 20 hours post injection, suggesting that the SPIO-protein/peptide complex may be stable at given physiological conditions. Animals were under observation during experiments and afterwards. No toxic effect from the agent was observed.

References: [1] Sundstrom JB, Mao H., et al. *J Acquir Immune Defic Syndr*. 2004; 35:9. [2]. Gao X, Cai H,et al: *Nat Biotechnol*. 2004; 22:969.



Scheme Illustration of IO-svFc EGFR complex. An EM photo of 4 nm IO nanocrystals is inserted.

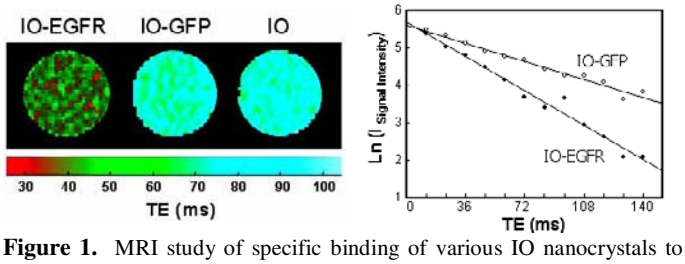


Figure 1. MRI study of specific binding of various IO nanocrystals to breast cancer cells (MDA-MB-231, EGFR+). Color coded panels showed IO-ScFv EGFR complex exhibited the greatest binding to EGFR+ cells evidenced by significantly shortened T2. Both wide-type IO nanocrystals and IO-GFP showed minimal T2 changes, indicating no specific binding to EGFR+ cells. T2 relaxation times were measured voxel-wise using a spin echo method at 3T and fitting signal decay exponentially over 12 TE points.

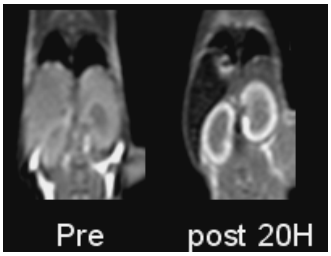


Figure 2. Comparison of spin-echo MRIs of pre and post intravenous injection of IO-scFv EGFR complex in a mouse showed signal drops in liver and spleen and signal enhancement of renal cortex 30 min. to 20 hrs after injection.