MR Molecular Imaging of Her-2/Neu Receptor with Gd based targeted Contrast Agent

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ABSTRACT:
The Her-2/*neu* cell surface receptor is important in breast cancer prognosis, and as a target for immunotherapy with monoclonal antibodies (Herceptin). We developed a specific Gd-based contrast agent for *in vivo* MR imaging of Her-2/*neu* receptors. Receptors are prelabeled with biotinylated monoclonal antibody and MR contrast is generated by probing the antibody with Gd-labeled avidin. Results demonstrate efficient delivery of the contrast agent to the tumor interstitium and a significant generation of *in vivo* contrast in animal models of breast cancer. The method can be used for assessing Her-2/*neu* status of tumors and for monitoring efficiency of anti-Her-2/*neu* immunotherapy.

INTRODUCTION:
Her-2/*neu*, a member of the epidermal growth factor receptor family c-erbB, is overexpressed in approximately 25% of breast cancers and other forms of human cancer. Overexpression of the receptor is associated with increased rates of relapse and death of breast cancer patients, and with other adverse prognostic factors. The Her-2/*neu* receptor is also an attractive target for immunotherapy with humanized monoclonal antibody (mAb) Herceptin, which shows efficacy against Her-2/*neu* positive breast tumors when used alone or in combination with cytotoxic chemotherapy [1]. Thus noninvasive molecular imaging of the Her-2/*neu* receptor can be immensely useful for prognosis, identification of tumors for Herceptin therapy, and to follow changes in the receptor status during treatment. Previously we tested a pretargeted contrast agent (CA) based on a combination of biotinylated primary mAb and streptavidin-linked SPIO nanoparticles [2]. This approach resulted in an efficient labeling of Her-2/neu expressing cell in cell culture. However poor delivery of the nanoparticles to tumors *in vivo* resulted in limited contrast enhancement for animal tumor models. Here we report the development of a new generation of a targeted MR contrast agent based on Gd-conjugated avidin protein which has a very high affinity to biotins on biotinylated primary antibody. The relatively small molecular size of both components of the CA results in efficient delivery to the tumor. The positive T_1 contrast generated by Gd is advantageous for *in vivo* applications.

METHODS:
Contrast agent: Purified avidin (O.E.M Concepts, NJ) was conjugated with DTPA and labeled with Gd ions according to standard protocols for protein modification (Pierce, IL). Efficiency of labeling (~9 GdDTPA groups per avidin) was estimated from T_1 relaxation measurements using albumin-GdDTPA conjugate as a reference.
Monoclonal antibodies: Commercial anti-rat Her-2/*neu* mouse mAb (Ab-9 Clone B10) were obtained from Neomarkers, CA. Biotin concentration, measured with HABA colorimetric assay, was 7.5 moles-biotin/mole-protein. Specificity of mAb was determined by immune fluorescence microscopy and FACS analysis. For *in vivo* applications mAb were purified from sodium azide preservative by superfiltration.
Animal Models: NT-5 Her-2/*neu*-rat expressing tumor cells were obtained from spontaneous breast cancers in transgenic mice and were grown subcutaneously in female SCID mice. The EMT-6 murine mammary carcinoma was used as a negative control. All animals received biotin-mAb (0.2ml i.v.), followed 12h later with 0.2ml of 60mg/ml avidin-GdDTPA administered i.v. in saline.
MRI: MR studies were performed on a 4.7T GE Omega spectrometer with a home built volume resonator. Animals were anesthetized with a mixture of ketamine/acepromazine (i.p). Multislice T_1-weighted images were acquired with a short TE spin-echo sequence.
Optical Detection: Fluorescent microscopy of cells and fresh tissue slices was used to assess efficiency of labeling and delivery of FITC-labeled avidin. Expression of the receptor was determined by Western Blot and flow cytometry (FACS) analysis with FITC-labeled antibody.

RESULTS:
Her-2/*neu* expression: Western blot protein assay and a histogram from FACS analysis of NT-5 and EMT-6 cells are shown in Fig.1 Significant expression of the receptor was observed for NT-5 cells and tumors and significantly lower expression was found in normal tissue and in EMT-6 cells.
Delivery of Avidin-FITC: Fluorescent microscopy slides of NT-5 tumor and liver slices shown in Fig.2 demonstrate an efficient delivery of FITC-labeled avidin molecules to tumor interstitium which was comparable to that for the liver.

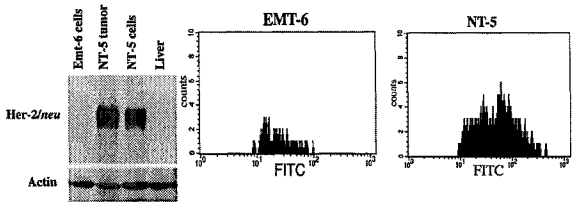

Figure 1. Western Blot and FACS analysis of Her-2/*neu* in NT-5 and EMT-6 tumors

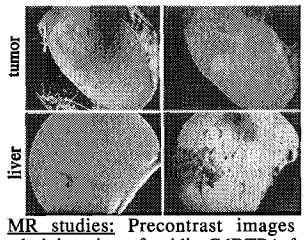

Figure 2. Delivery efficiency of avidin-FITC conjugate detected by fluorescent microscopy of fresh tissue sections obtained from the liver and NT-5 tumor 1h after i.v. injection of the compound. Fluorescent microscopy was obtained at a wavelenght of 512nm using a Nikon TS100-F microscope (1x objective).

MR studies: Precontrast images and images obtained 22h after administration of avidin-GdDTPA for NT-5 and EMT-6 bearing animals are shown in Fig.3. Strong positive T_1 contrast enhancement was detected for NT-5 tumors where specific binding of mAb significantly prolonged retention of avidin-GdDTPA.

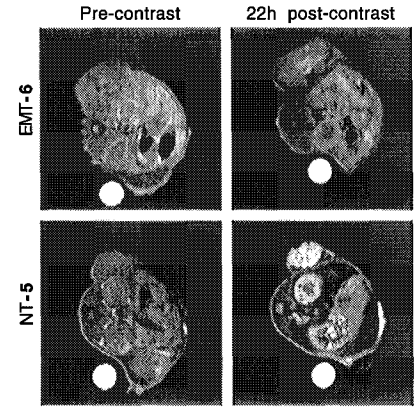

Figure 3. T_1 weighted pre- and post-contrast images of NT-5 and EMT-6 tumors grown in SCID mice. MR imaging was performed with following parameters: TE/TR=16/300ms, FOV of 32mm, ST of 2mm, 256x128 matrix, 4 averages. Avidin-GdDTPA contrast agent was administered 12h after injection of biotinylated mAb.

DISCUSSION:
We detected a significant positive T_1 contrast generated by Avidin-GdDTPA in tumors prelabeled with a specific biotinylated mAb.. Expression of the target receptors in the model tumors and delivery of the contrast agent to the tumor were independently established by different techniques. The relatively small molecular weight of both components of the targeted contrast agent (150kD for mAb and 70kD for avidin-GdDTPA) results in efficient delivery to the tumor interstitium compared to magnetic nanoparticles. Positive signal enhancement is another major advantage for *in vivo* molecular MR imaging. To evaluate the detection limit for this method we can assume the number of Her-2/*neu* receptors per cell to be in the range of 10^5 - 10^6. If each receptor is labeled with mAb and each mAb is loaded with 7 molecules of avidin, (9 Gd per avidin) the estimated concentration of gadolinium is ~10^7 atoms per cell. Recalculating for the cell volume of 10^{-9}ml we obtain Gd concentration in the range of 16μM. Several factors will influence the efficiency of the method, including stability of antibody-receptor complex on the surface of cell membrane, blocking of injected avidin by endogenous biotin, and clearance of unbound contrast agent. Therefore, to provide maximum sensitivity, the experimental protocol as well as the doses of both components of the contrast agent have to be carefully optimized.

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