Feasibility of cNGR labeled paramagnetic quantum dots for molecular Magnetic Resonance Imaging and Two Photon Laser Scanning Microscopy of neovascularization

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Introduction

Angiogenesis, the formation of new capillaries out of existing blood vessels, plays an important role in many pathologies. Angiogenic activity is, for instance, observed in tumor growth, the formation of metastases or chronic inflammatory diseases. Impaired neovascularization is often found in patients suffering from myocardial infarction (MI) or peripheral artery occlusive disease[1]. Hence, the detection of angiogenesis, with for example molecular Magnetic Resonance Imaging (MRI), is important for the diagnosis and treatment follow-up of these conditions.

CD13, a transmembrane glycoprotein likely involved in chemokine processing and tumor invasion, is strongly upregulated on the endothelial cells of vessels undergoing neovascularization[2]. Its spatial distribution may be visualized using contrast agents labeled with the cyclic asparagine-glycine-arginine (cNGR) peptide, a proposed ligand for CD13[3]. Here, we demonstrate the applicability of cNGR labeled bimodal, i.e. paramagnetic and fluorescent, quantum dots (QDs) for the non-invasive, direct and selective detection of angiogenesis in tumors and infarcted myocardium using molecular MRI. Results were validated using Two Photon Laser Scanning Microscopy (TPLSM), which allowed a more detailed localization of the contrast agent.

Methods

Contrast agent Streptavidin coated Cadmium/Selenium quantum dots (585 nm emission) were purchased from Invitrogen (Breda, The Netherlands). CD13 specificity was introduced via biotin-cNGR, which was synthesized analogously to the methods described by Dirksen *et al*[4]. Biotin-Gd-DTPA-wedge, containing 8 Gd-DTPA molecules per poly(lysine) dendritic wedge, was used to obtain sufficient MRI contrast[5]. The final contrast agent (henceforth called cNGR-QD-Gd:wedge, Fig 1) was obtained by mixing QDs, biotin-cNGR and biotin-Gd-DTPA-wedge in a molar ratio of 1:6:24.

Tumor angiogenesis Male athymic Swiss mice received a subcutaneous injection of ~$1.5x10^6$ human colon carcinoma cells (LS174T) on the right flank. Tumors grew for approximately 14 days and had a size of ~1.5 cm^3 at the day of MRI. Experiments were performed on a Philips Intera 1.5T clinical scanner (Philips Medical Systems, Best, The Netherlands). Pre-contrast T_1 values were determined using a series of inversion recovery (IR) measurements with increasing inversion times. Next, 100 µL of a 1 µM contrast agent solution was injected into the tail vein and changes in the T_1 relaxation rate (R_1) were calculated for voxels showing a signal increase of at least 3 times the noise level, using pre and post contrast high resolution IR images (voxel size 0.36x0.36 mm^2, 1.5 mm slice thickness, TI 800 ms). After MRI, tumors were excised and incubated in endothelial cell specific αCD31-FITC for investigation by TPLSM.

Myocardial infarction angiogenesis MI was induced in male Swiss mice by ligating the left descending coronary artery, as described previously[6]. MRI experiments were performed 7 days after surgery on a Bruker Biospec 7.0T scanner (Bruker Biospin GmbH, Ettlingen, Germany). Contrast agent was administered as described above. Pre and post contrast black blood images were obtained using a respiratory gated and cardiac triggered FLASH black blood module (voxel size 0.12x0.12 mm^2, 1.14 mm slice thickness, TI 80 ms). *Ex vivo* TPLSM validation measurements on the excised heart were performed as described for the tumor study.

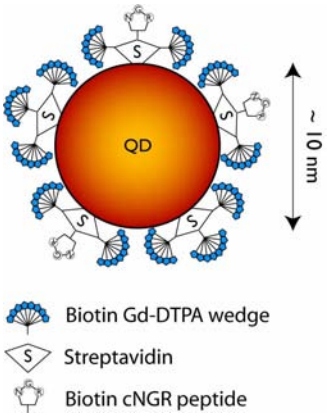

Figure 1 *cNGR-QD-Gd:wedge*

Results

Tumor angiogenesis Upon the injection of cNGR-QD-Gd:wedge, 35% of the tumor voxels showed a significant signal increase, leading to an R_1 increase in the entire tumor of 5% (Fig 2a). In contrast, using control QDs (without ligand), the signal was increased in only 7% of the tumor voxels with a corresponding R_1 increase of just 1% (Fig 2b). In (non-angiogenic) muscle tissue, a signal increase was observed in approximately 10% of the voxels after targeted contrast agent injection, which is likely due to a blood volume effect and not due to specific ligand-receptor interactions. TPLSM measurements showed that cNGR-QD-Gd:wedge was localized in both the vessel lumen and the interstitial space of the tumor tissue (Fig 2c). Furthermore, control QDs were not detected in the tumor tissue using TPLSM (Fig 2d).

MI angiogenesis Signal intensity changes were calculated for the infarct region using pre and post contrast black blood images. As is depicted in Figure 3a, the infarct area is readily distinguished from the surrounding healthy tissue. Next, TPLSM images were acquired from both the infarcted and healthy myocardium (Figs 3b/c, respectively). cNGR labeled contrast agent was only detected in the infarct area.

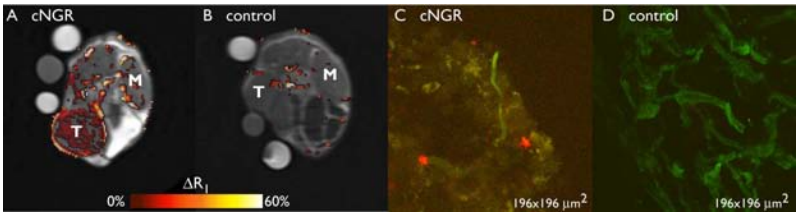

Figure 2 *A/B) T_1 weighted IR images with overlay of R_1 increase. T: tumor, M: muscle. C/D) TPLSM images of tumor tissues. Green αCD31-FITC (endothelium), Red: cNGR-QD-Gd:wedge*

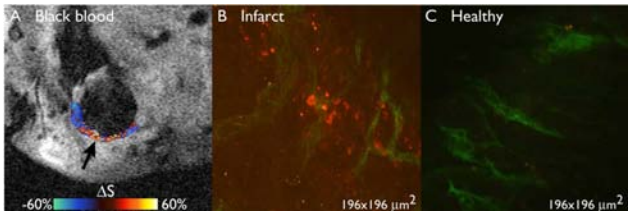

Figure 3 *A) End-diastolic black blood image with overlay of signal changes. The infarct area is indicated with an arrow. B/C) TPLSM images. Green: αCD31-FITC (endothelium), Red: cNGR-QD-Gd:wedge*

Conclusions

cNGR-QD-Gd:wedge was demonstrated to be a suitable probe for the non-invasive detection of neovascularization with molecular MRI. In both tumor and MI models, angiogenic areas showed a significant signal enhancement due to the contrast agent. In contrast, the injection of untargeted QD-Gd:wedge resulted in almost no signal increase in the tumor model, thereby indicating a high specificity of cNGR for angiogenic endothelial cells. The specificity of the cNGR labeled contrast agent for angiogenic areas found in this MRI study is in good correspondence with results obtained by *in* and *ex vivo* fluorescence microscopy[7]. The excellent fluorescent properties of QDs furthermore allowed a rapid and easy validation of the MRI results using *ex vivo* TPLSM. Currently, we are increasing the number of animals to be investigated and are determining the T_1 and T_2 relaxivities of the probe.

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