Parallel intravital microscopy, MR imaging, and fluorescence imaging of tumor angiogenesis using paramagnetic quantum dots

W. J. Mulder[1,2], K. Castermans[3], C. W. Lowik[4], E. L. Kaijzel[4], G. J. Strijkers[2], A. W. Griffioen[3], and K. Nicolay[2]

[1]Department of Radiology, Mount Sinai School of Medicine, New York, New York, United States, [2]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [3]Angiogenesis Laboratory, Department of Pathology, Maastricht University, Maastricht, Netherlands, [4]Department of Endocrinology and Metabolic Diseases (C4-R), Leiden University Medical Centre, Leiden, Netherlands

Introduction

Multimodality molecular imaging is a promising and refined technique to non-invasively visualize tumor angiogenesis, which has so far been largely unexplored due to the lack of suitable multimodal contrast agents. Here we report on the application of a novel αvβ3-specific quantum dot based nanoparticle (RGD-pQDs), which has been optimized for both optical and magnetic resonance (MR) detection of tumor angiogenesis[1]. The utility of this contrast agent for multimodality molecular imaging was evaluated on tumor bearing mice. Upon intravenous injection of RGD-pQDs intravital microscopy (IVM) allowed the investigation of angiogenically activated endothelium at cellular resolution with a small scanning window and limited penetration depth, while MRI was used to visualize angiogenesis sites at anatomical resolution throughout the entire tumor. Fluorescence imaging allowed the investigation of angiogenic activity of the entire mouse with high sensitivity and with much shorter scan times than was the case for both previous techniques.

Material and Methods

QDs with a paramagnetic lipidic coating were synthesized according to a method described previously[1, 2]. Multiple cyclic RGD ligands were covalently linked to the lipidic coating[3]. Bare QDs were used as a non-functional control agent.

IVM and MRI of angiogenically activated vessels were employed on 10 C57Bl6 mice inoculated with B16F10 melanoma cells on the flank. For IVM the tumor was prepared free by removing the skin. RGD-pQDs or non-specific pQDs were injected intraveneously and visualized using a Leitz intravital microscope adapted for telescopic imaging. For *in vivo* MRI mice were anesthetized and an infusion line with the contrast agent was brought into the tail vein. Animals were placed in a 6.3 T MRI scanner. High resolution T1-weighted images (TR 800 ms, TE 10 ms) were generated prior to and after administration of the contrast agent. The MR-data were analyzed with Mathematica.

Whole body fluorescence imaging was done on Balb/c nude mice inoculated with luciferase (luc) expressing human renal carcinoma cells (RC21-luc). Ten weeks after inoculation, luciferase expression was assessed 5 minutes after intraperitoneal (i.p.) injection of luciferin. Whole body fluorescence imaging of both RGD-pQDs and luciferase in vivo was accomplished using a peltier cooled CCD camera system NightOWL "UltraSense Frontlit" LB 981. Mice were anaesthetized and placed in the imaging cabinet and images were acquired 10 minutes after intracardial injection of RGD-pQDs at a camera exposure time of 10 seconds. Fluorescent and luminescent signals were expressed in photons/cm2/s by using the WinLight 32 software (Berthold Technologies).

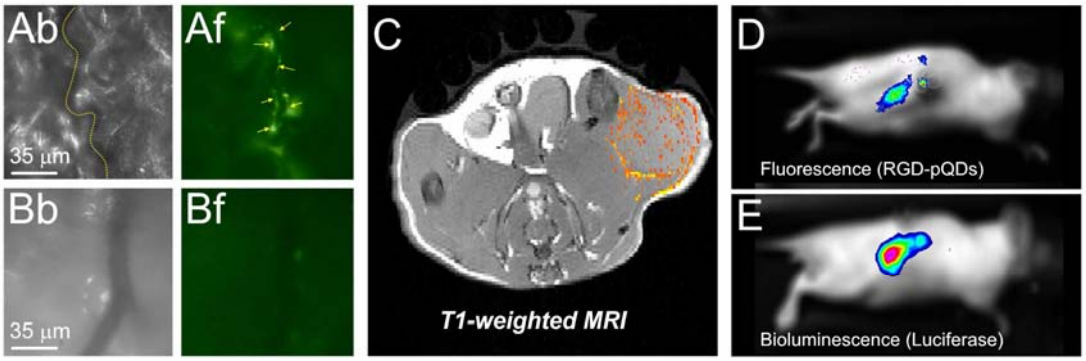

Figure *Images of animals injected with RGD-pQD.*

Bright field images (Ab, Bb) and fluorescence images (Af, Bf) after injection of the tumor vasculature (A) and of resting vasculature (B). MR image with color coded signal enhancement in the tumor (C). Whole body fluorescence image (D) shows co-localization of QD signal with luciferase expression (E).

Results and Discussion

Intravital microscopy revealed association of RGD-pQDs with activated endothelium of tumor blood vessels. In Figure A a bright field image (Ab) and a fluorescence image (Af) after injection of the contrast agent is depicted. The activated endothelial cells and thus the contours of the blood vessel are indicated with yellow arrows. No association of RGD-pQDs with resting endothelial cells was found in the ears of mice (Figure B). Furthermore, no labeling of either tumor or resting endothelium was observed upon injection of the non-specific agent (not shown). T1-weighted MR images generated before and 45 minutes after the injection of the RGD-pQDS were used to measure the signal enhancement in the tumor. In Figure C pixels in the tumor with signal enhancement of at least five times the noise level are color coded according to the pseudo-color scale: 0 40% signal enhancement and revealed the highest angiogenic activity in the periphery of the tumor.

Fluorescence imaging on Balb/c mice, 10 minutes after intracardial injection of RGD-pQDs, revealed a strong fluorescent signal (Figure D), which co-localized with the signal that originated from the luciferase expression site (Figure E). This finding again demonstrates the specificity of the QD targeting.

Conclusions

This study demonstrates molecular imaging of angiogenesis after intravenous administration of targeted quantum dots specific for the αvβ3-integrin using a multimodality approach. In a parallel fashion highly complementary imaging techniques, namely intravital microscopy, MRI, and whole body fluorescence imaging were applied on live animals. This approach is valuable for the investigation of the angiogenic process and the development of anti-angiogenic therapies. The present nanoparticle may also be used for multimodality molecular imaging of other pathophysiological processes[4].

References

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