Multimodality molecular imaging of tumor angiogenesis using quantum dots

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Introduction

Quantum dots, semiconductor nanocrystals in a size range of 2 to 6 nm have gained much interest the last few years for biological imaging purposes[1], especially because of their bright fluorescence, their photo-stability, and their narrow and tunable emission spectrum. In this study we developed quantum dots (QDs) with a pegylated and paramagnetic coating (Figure 1A) to make them detectable by both MR and optical imaging. Angiogenesis, the formation of new blood vessels, is involved in many pathological processes, including cancer[2] In the angiogenic cascade different cell surface receptors, including the αvβ3-integrin, are expressed at the activated tumor endothelium. The non-invasive *in vivo* detection of this integrin would thus allow one to monitor angiogenesis and to follow the effect of anti-angiogenic therapies. To that aim multiple αvβ3-specific RGD-peptides were conjugated to the paramagnetically coated quantum dots (RGD-pQDs). Next, the RGD-pQDs were assessed for their specificity on endothelial cells (HUVEC) *in vitro*. Cells that were incubated with bare pQDs and cells that were incubated without contrast agent were used as controls. On the cells both fluorescence microscopy and MRI was performed.

Upon injection of this contrast agent activated endothelial cells were visualized in tumor bearing mice with intravital microscopy (IVM) and MRI *in vivo*. The assessment of the tumors with IVM allowed the investigation of angiogenesis at subcellular level with a small scanning window and limited penetration depth. On the other hand, MRI allowed the investigation of angiogenesis on anatomical level with a large scanning window.

Material and Methods

High-quality CdSe/ZnS core/shell QDs capped with TOPO/HDA were synthesized. A micellular and paramagnetic lipidic coating was applied according to an extended method described previously[3]. The cyclic 5mer RGD was conjugated to maleimide-PEG-DSPE incorporated in the lipidic coating[4]. The specificity of the RGD-pQDs was determined *in vitro* on HUVEC expressing αvβ3 with MRI and fluorescence microscopy.

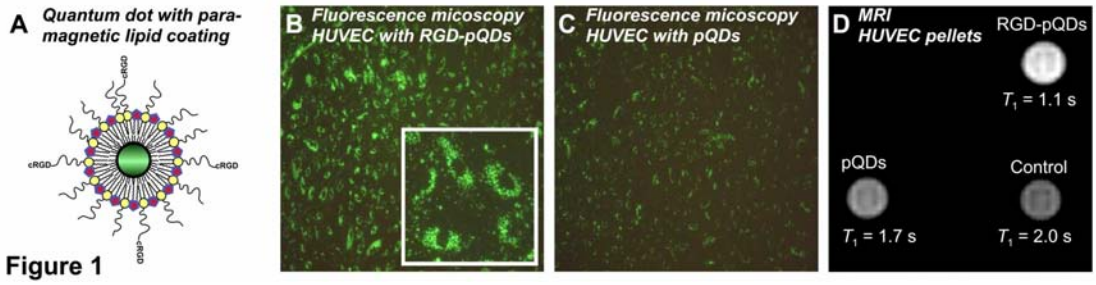

Figure 1

For *in vivo* MRI and IVM mice (n=4) were anesthetized and an infusion line with the contrast agent was brought into the tail vein. Next, the mice were either assessed with IVM or MRI. For IVM the tumor was prepared free by removing the skin. RGD-pQDs were visualized using a Leitz intravital microscope adapted for telescopic imaging. For MRI mice 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.

Results and Discussion

In vitro, both MRI and fluorescence microscopy revealed a strong association of the RGD-pQDs with αvβ3 expressing HUVEC (Figure 1B and D), while non-targeted pQDS associated to HUVEC in a much smaller extent (Figure 1C and D).

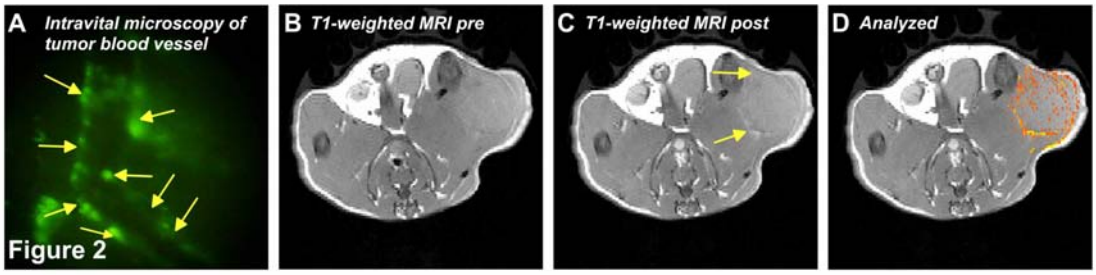

Figure 2

Intravital microscopy revealed association of RGD-pQDs with activated tumor endothelium of tumor blood vessels. In Figure 2A the activated endothelial cells and thus the contours of the blood vessel are indicated with yellow arrows. Association of RGD-pQDS with vessels was found as far as 0.5-1.0 cm from the tumor boundary. No association of RGD-pQDs with endothelial cells was found in the ears of mice. T1-weighted MR images measured before (Figure 2B) and 20 minutes after (Figure 2C and D) the injection of the RGD-pQDS showed the accumulation of the contrast agent in the tumor. The arrows in Figure 2C indicate a bright region appearing at the periphery of the tumor. In Figure 2D 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 30% signal enhancement.

Conclusions

The technology developed in this study allows multimodality molecular imaging of angiogenesis by using targeted quantum dots specific for the αvβ3-integrin in combination with intravital microscopy and MRI. Both imaging techniques were applied on live animals and are highly complementary, which is very desirable for the investigation of the angiogenic process.

References

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