Tumor vasculature labeling by αvβ3-targeted liposomes. A combined MRI and fluorescence microscopy study.

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Introduction

Angiogenesis, the formation of new blood vessels, is involved in many pathological processes, including cancer[1]. Inhibition of angiogenesis is a novel strategy to stop tumor growth/progression. In the angiogenic cascade different cell surface receptors, including the αvβ3-integrin, are expressed at the endothelium of the new vasculature. 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. Several studies have used the tripeptide sequence arginine-glycine-aspartic acid (RGD) conjugated with a radiolabel to image αvβ3 expression with PET[2], and Scintigraphic Imaging[3]. Recently paramagnetic nanoparticles conjugated with RGD were successfully used to visualize angiogenesis in rabbit tumors with MRI[4].

In the present study, we aimed to image angiogenesis by detecting the expression of αvβ3 in tumor bearing mice with both MRI and fluorescence microscopy. MR-detectable and fluorescent liposomes[5], which carry over 300 RGD-moieties per liposome, were used. RAD-conjugated liposomes were used as a non-specific control. We used liposomes, since they can carry a high pay-load of a lipid-based MRI contrast agent. The *in vivo* MRI findings were validated with *ex vivo* fluorescence microscopy.

Material and Methods

Liposomes containing Gd-DTPA based lipid and Rhodamine-PE were prepared as described previously[5]. The cyclic 5mer RGD (c(RGDf(-S-acetylthioacetyl)K) and control RAD-peptide (c(RADf(-S-acetylthioacetyl)K) were conjugated to maleimide-PEG-DSPE incorporated in the liposomes, after deacetylation of the thioacetyl group. The specificity of the liposomes was determined *in vitro* on HUVEC expressing αvβ3 with MRI and fluorescence microscopy.

Tumor bearing mice were anesthetized with an isoflurane/air mixture and an infusion line was brought into the tail vein. Next, the mice were placed in a 6.3 T MRI scanner. A T1-map and a T1-weighted image were generated prior to administration of the contrast agent. After the contrast agent was injected every 35 minutes a T1-weighted image was generated for at least 5 time points. To assess specificity a competition experiment was performed with non-paramagnetic RGD-liposomes to block the αvβ3-integrin, followed by the administration of paramagnetic RGD-liposomes. Upon completion of MR scanning, the mice were euthanized and the tumor was dissected and frozen in isopentane. Sections of tissue (10-µm thickness) were prepared, nuclei were stained by addition of DAPI, and fluorescence microscopy was performed. All MR-data were analyzed with Mathematica.

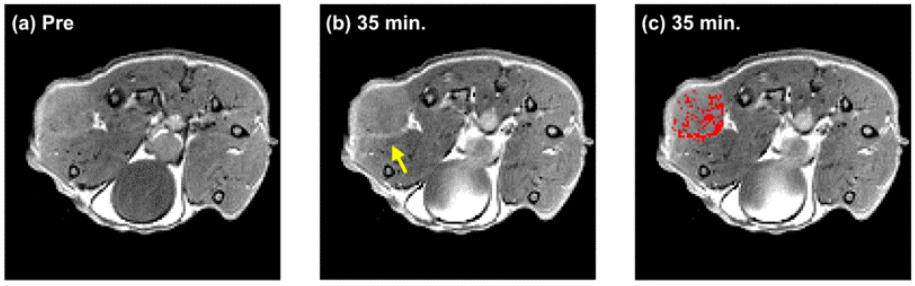

Figure 1: (a) Pre and (b,c) 35 min. post contrast images. The red dots in (c) indicate those pixels in the tumor with a significant signal intensity increase.

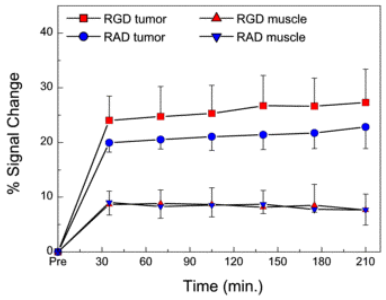

Figure 2: Signal intensity changes of tumor and muscle using RGD- and RAD-conjugated liposomes.

Results and Discussion

Both MRI and fluorescence microscopy revealed a strong association of the RGD-liposomes with αvβ3 expressing HUVEC, while RAD-liposomes did not. MRI on tumor bearing mice showed contrast enhancement of the tumor after injecting the αvβ3-specific contrast agent (figure 1b arrow). Both RGD-liposomes and RAD-liposomes showed contrast enhancement in MR images, with a somewhat larger signal increase for the RGD-liposomes compared to the RAD-liposomes (figure 2). Mice that were first injected with non-paramagnetic RGD-liposomes, followed by injection of paramagnetic RGD-liposomes, did not show signal changes in the tumor. Fluorescence microscopy revealed a distinct difference between tumors of mice that were injected with RGD-liposomes or RAD-liposomes. In case of RAD-liposomes a diffuse pattern of fluorescence was found around blood vessels within the tumor tissue (figure 3a), indicative of extravasation of the liposomes. In contrast, RGD-liposomes were exclusively found within blood vessels (figure 3b), suggesting a specific association with αvβ3 expressed at the angiogenic endothelium.

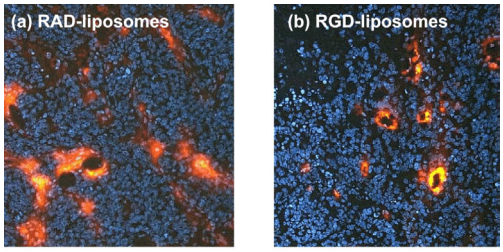

Figure 3: Fluorescence microscopy of (a) RAD- and (b) RGD-liposomes in the tumor tissue.

Conclusions

This study demonstrates that molecular MR imaging of angiogenesis is possible by using a targeted contrast agent specific for the αvβ3-integrin, and that proper microscopic validation is needed for interpreting the MRI findings.

References

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