αvβ3 -targeted nanoemulsions for tumor angiogenesis phenotyping with MRI and NIRF imaging

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Introduction: The angiogenic level of a malignancy correlates with its aggressiveness and is an important parameter for the prognosis in cancer therapy. New, noninvasive imaging methods and contrast agents are therefore highly desirable and increasingly explored to improve the accuracy of the assessment of tumor angiogenic activity. Certain cell surface receptors, including the αvβ3 integrin, are highly overexpressed during the formation of new tumor blood vessels and can be used for active nanoparticle targeting. In the current study the RGD-peptide, which has a high affinity for the αvβ3 integrin, was attached to the surface of a nanoparticle platform we described previously (1). It is based on oil-in-water nanoemulsions with a tunable particle mean size in a range of 25-100 nm, and the possibility to include lipophilic contrast agents in the core as well as amphiphilic ones in the corona. Here, we focused on iron oxide enhanced T2/T2*-weighted MRI and near infrared fluorescence (NIRF) imaging. Two different nude mouse tumor models with different microvessel densities (MVD)/angiogenesis levels were used to test the potential of our platform as a probe for multimodal *in vivo* angiogenesis imaging and tumor phenotyping.

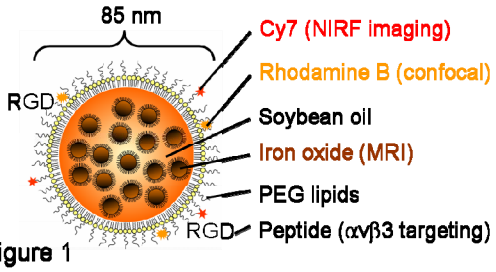

Figure 1

Materials and Methods: Nanoemulsions were synthesized by a lipid film-method followed by sonication. They were comprised of DSPC, PEG-DSPE, Cy7-PEG-DSPE for NIRF imaging, PEGylated lipids with a distal maleimide group (Mal-PEG-DSPE) for functionalization with cyclic RGD, Rhodamine B-phospholipids (for confocal microscopy) and soybean oil. Oleic acid coated iron oxide nanocrystals (10 nm) were suspended in the oil core to enable T2/T2* weighted MRI (Figure 1). Two different subcutaneous swiss nude mouse models were used: LS174T (human colon carcinoma) and EW7 (human Ewing's sarcoma). For the *in vivo* MRI experiments, five groups of mice were used with 6 mice per group: 1) LS174T RGD, 2) LS174T control (injected with untargeted particles), 3) EW7 RGD, 4) LS RGD (for competition experiment, one injection), 5) LS174T comp (two injections: pre-injected with particles lacking iron oxide). The animals were pre-scanned with a 9.4 T dedicated small animal MRI system, injected with a dose of 36 mg/kg Fe and continuously scanned up to 4 h post injection without changing the position of the mouse. For NIRF imaging three mouse groups were included: 1) LS174T RGD, 2) LS174T control 3) EW7 RGD. To study the nanoparticle accumulation kinetics mice were scanned 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h and 26 h post-injection (Figure 2). After the last scan, mice were sacrificed and organs as well as tumors were excised to allow the determination of biodistribution with fluorescence imaging of intact tissues, as well as to allow histological analysis using Perl's staining and confocal laser scanning microscopy (CLSM) of tissue sections.

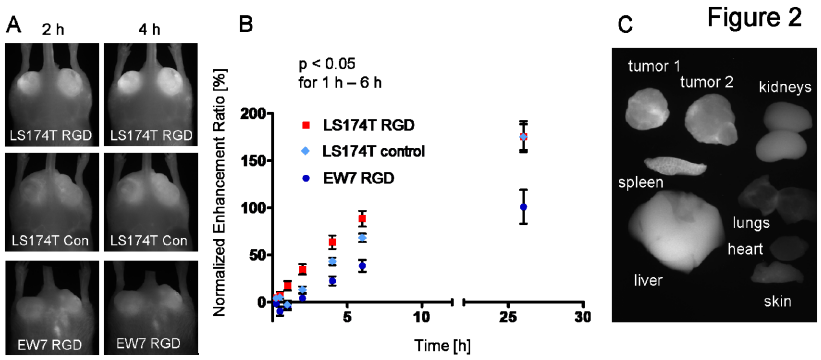

Figure 2

Results and Discussion: The analysis of the NIRF images (Figure 2A, examples shown for 2 h and 4 h) of the mice injected with αvβ3-targeted nanoemulsions (mean diameter 85 nm) revealed a statistically significant ($p<0.05$) difference for the two different tumor models for the time points 1 h, 2 h, 4 h and 6 h (Figure 2B). Ex vivo histological examination of iron oxide as well as CLSM showed that the targeted particles were localized at the endothelium of tumor blood vessels, whereas control particles could be found predominantly in the interstitial space. Moreover, fluorescence images of the excised organs and the tumors showed a high accumulation level of the targeted particles in the tumors close to the level of that found in the liver (Figure 2C). Different patterns of hypointense signal, and hence biodistribution of the nanoemulsions, could be observed in the *in vivo* MRI post-scans (T2*-w: TR 120 ms, TE 3 ms, 30° flip angle) between the two tumor models (LS174T vs EW7). In the LS174T RGD group, signal attenuation was primarily confined to the periphery of the tumor (Figure 3), where angiogenesis activity is highest, compared to the homogeneous distribution observed in the three other groups (EW7 RDG, LS174T control group injected with untargeted particles and LS174T competitive inhibition).

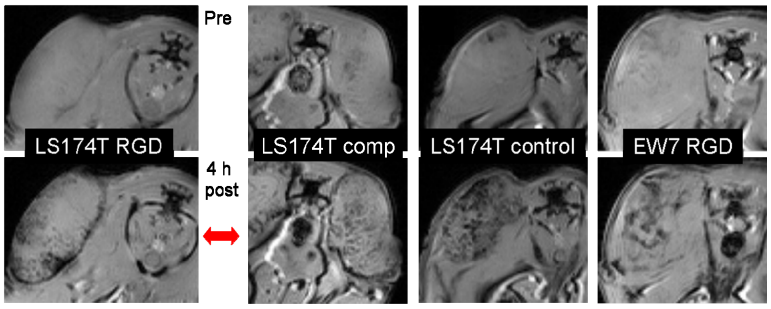

Figure 3

Conclusions: In conclusion, we were able to synthesize stable, αvβ3 -targeted nanoemulsions carrying iron oxide nanocrystals in the core for MRI and fluorophores (Cy7, Rhodamine B) attached to the surface for optical imaging. Using this nanoparticle platform we could distinguish the angiogenesis activity and distribution in two different tumor mouse models with known differences in angiogenesis activity with a combination of in vivo MRI and NIRF imaging.

References: (1) Jarzyna et al. Biomaterials. 2009.