Reconstituted high density lipoprotein nanoparticles for multimodality molecular imaging of tumors

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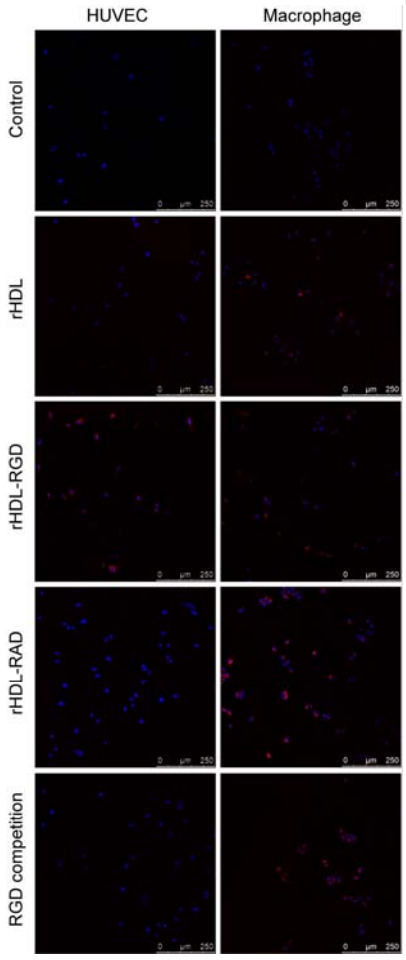
Introduction: High density lipoprotein (HDL) has been modified previously to function as a versatile nanoparticle platform for magnetic resonance molecular imaging of atherosclerosis (1,2). HDL naturally targets macrophages in e.g. atherosclerotic plaques and the liver. Macrophages also play an important role in tumors. These so-called tumor-associated-macrophages (TAM) are involved in the promotion of angiogenesis, matrix remodeling and suppression of adaptive immunity (3). In the present study, we utilize the aforementioned HDL nanoparticles platform for tumor imaging by targeting them naturally to macrophages and by rerouting them to endothelial cells in angiogenically activated blood vessels.

Methods: The discoidal rHDL nanoparticles were synthesized as previously reported (1,2) with a Gd-DTPA and a fluorescent lipid incorporated. The latter was either Rhodamine-PE or a near infrared (NIR) dye DiR. The rHDL nanoparticles were further functionalized with αvβ3-specific cyclic RGD peptides (rHDL-RGD) through the exposed lysine units of apoA-I (the major protein of HDL). Nonspecific cyclic RAD peptides were conjugated to rHDL (rHDL-RAD) as a control.

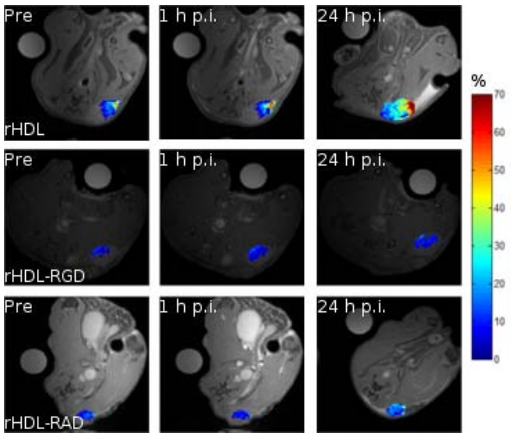


Murine J774A.1 macrophages and human umbilical vein endothelial cells (HUVEC) were cultured and were grown to 60-70% confluent before incubation with nanoparticles at 0.05 mM [Gd]. The association of the particles with the cells was assessed using fluorescence microscopy.

To generate tumor xenografts, EW7 Ewing sarcoma cells were injected subcutaneously into the right lower back of the swiss nude mice. Between day 21 and 28, when tumors had grown to a diameter of 4-5 mm, mice were used for imaging. For in vivo MR imaging, mice were anesthetized and an infusion line with the contrast agent was brought into the tail vein. Animals were placed in a 9.4 T MRI scanner. High resolution T1-weighted images (TR=800 ms, TE=10 ms) were generated pre- and post-injection of the contrast agent. A whole tumor based intensity analysis was used to determine the signal enhancement by a Matlab procedure. For in vivo NIR imaging, mice were anesthetized and intravenously administrated with nanoparticles. NIR images were with a 790 nm excitation and 800 nm emission filter.

Results and Discussion: Confocal microscopy images were used to assess the association of nanoparticles with cells in vitro (left figure). In vitro, native rHDL, rHDL-RGD and control rHDL-RAD nanoparticles exhibited affinity for murine J774A.1 macrophage cells. On the other hand rHDL-RGD nanoparticles showed increased uptake affinity for αvβ3-expressing HUVEC when compared to rHDL and rHDL-RAD. This specific uptake was further confirmed by a competitive inhibition experiment.

In vivo, rHDL, rHDL-RGD, and rHDL-RAD nanoparticles gave rise to signal increase of tumors on *T*1-weighted MR images (right figure). The enhanced fractions of tumors were not significantly different between the administration of rHDL, rHDL-RGD, and rHDL-RAD nanoparticles. However, using NIR imaging it was observed that the tumors of mice injected with rHDL-RGD showed rapid signal increase, which likely is due to the fast binding of rHDL-RGD nanoparticles to angiogenic endothelial cells. On the other hand, the rHDL enhancement of the tumor was more gradual. Immunofluorescent staining showed different accumulation patterns of rHDL, rHDL-RGD and rHDL-RAD nanoparticles in the tumors. The rHDL-RGD nanoparticles were associated with endothelial cells while rHDL and non-specific rHDL-RAD nanoparticles were colocalized with CD68+ macrophages.



Conclusions: We were able to apply rHDL as a multifunctional platform for in vivo tumor imaging by MRI and NIR as well as for ex vivo confocal imaging. The conjugation with αvβ3 target-specific peptides to rHDL nanoparticles enabled redirection of rHDL from its natural target of macrophages to endothelial cells of angiogenic tumor vessels.

References:

(1) Frias JC et al. J Am Chem Soc, 2004; (2) Frias JC et al. Nano Lett, 2006; (3) Mantovani A et al. Cancer Metastasis Rev, 2006