Image-guided delivery of liposomal nano-constructs targeting tumor vasculature

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Target audience: Scientists who are interested in image-guided drug delivery and cancer MRI/therapy.

Purpose: Noninvasive visualization of drug delivery and controlled release is highly beneficial in drug development and image-guided therapy. The image-guided cancer therapy associated with nano-constructs allows the prediction of the therapy outcome, which will eventually benefit cancer patients. Superparamagnetic iron oxide nanoparticles (SPIONs) are highly sensitive MRI contrast agents. However, use of SPIONs as an image-guided drug delivery platform is problematic because of rapid clearance of SPIONs by the reticuloendothelial system. In this study, we investigate if delivery of SPIONs to tumor can be enhanced by encapsulating them in liposomes for either "passive" delivery into tumor via the enhanced permeability and retention effect (EPR) or "active" delivery to tumor endothelium via the receptors for vascular endothelial growth factor (VEGF).

Methods: *In vivo* MRI of orthotopic MDA-MB-231/luc tumor xenografts was performed on a preclinical 9.4T MRI scanner following intravenous administration of either free (SPIONs) or non-targeted Lip(Gd/Fe) and VEGF receptor targeted scVEGF-Lip(Gd/Fe) liposomal SPIONs dual labeled with SPIO and GdDTPA to detect liposome degradation (Fig. 1A).[1] Lip(Gd/Fe) were designed for passive targeting strategy, i.e. liposomes contained PEGylated lipid to prolong systemic circulation, whereas scVEGF-Lip(Gd/Fe) contained PEG as a part of the chemical linker scVEGF-PEG$_{3400}$-DSPE. Microscopic uptake of fluorescence-labeled constructs in the tumor was detected *in vivo* by multiphoton intravital microscopy as shown in Fig. 1B.

Results: Representative *T2*-weighted MR images of the tumors before and after intravenous administration of free SPIONs and two liposomal formulations are shown in Fig.1C. *In vivo* MRI study revealed that at 4 and 24 hours post-injection non-targeted liposomal formulation of SPIONs provides improved tumor accumulation in comparison to SPIONs or liposomal formulations targeted to VEGF receptors. The uptake index, used as a measure of the tumor uptake of SPIONs, indicated that the highest uptake occurred 4 h after the administration in all three groups; however, only Lip(Gd/Fe) resulted in statistically significant hypo-intense tumor signals at 4 h time point (Fig. 1C). Visually detectable but statistically nonsignificant tumor uptake of SPIONs was detected in free SPIONs and scVEGF-Lip(Gd/Fe) experimental groups. Intravital microscopy images of the distribution of the fluorescent scVEGF probe in the tumor vasculature are shown in Fig. 1D, Panel 1. Complete clearance of the probe from the blood stream and binding to the blood vessel walls was detected 35 min after administration. The fusion of the probe with vascular dextran marker is shown in Fig. 1D, Panel 2. Intravital microscopy images of the distribution of control untargeted and scVEGF targeted fluorescent liposomes in the vasculature of MDA-MB-231 tumors are shown in Fig. 1E using 10x (top) and 25x (bottom) magnifications. High magnification images (bottom) demonstrate extravasation of the untargeted liposomes due to EPR effect (red arrow) whereas no extravasation was detected for the targeted liposomes presumably due to high-affinity binding to the endothelial cells via VEGFR2 receptors.

Discussion: Experimental data revealed that the delivery of SPIONs to MDA-MB-231 tumors was improved by encapsulating them into non-targeted and targeted liposomes. EPR effect associated with leaky tumor vasculature apparently provides better tumor accumulation of liposomal SPIONs than their presumably more transient retention via binding to VEGF receptors in the tumor vasculature. To explain the lower accumulation of VEGFR-targeted liposomes, we hypothesize that VEGFRs on tumor endothelium, in effect, act as a barrier for targeted liposomes and therefore interfere with EPR-mediated extravasation and accumulation. We also cannot exclude the possibility that decoration of liposomes with scVEGF could by itself change their pharmacokinetics and the ability to extravasate. In addition, blood vessels constitute only a few percent of the total tumor mass, and significant number of VEGFR-2 receptors on a tumor endothelial cell is reported only on a subset of those cells[2].

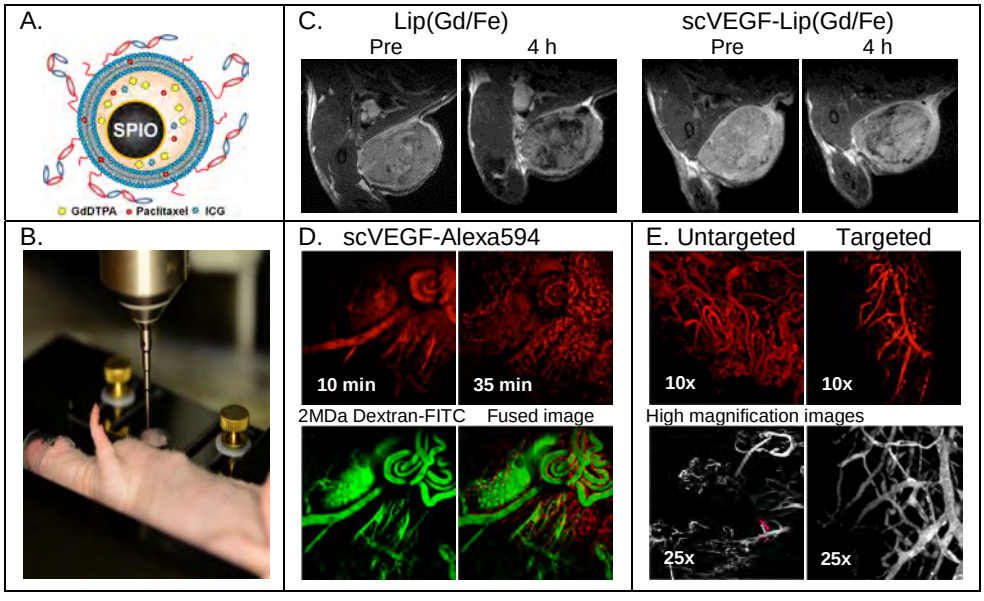

Figure 1. A. Schematics of dual magnetic labeled liposomal nano-constructs. **B.** Experimental setup for multiphoton intravital fluorescence microscopy in mouse tumor models. **C.** *T2*-weighted MR images of orthotopic MDA-MB-231/luc tumors before and after intravenous administration of untargeted and targeted liposomal nano-construct formulations. **D.** Intravital microscopy images of the distribution of fluorescent scVEGF probe in the tumor vasculature. Complete clearance of the probe from the blood stream and binding to the blood vessel walls was detected 35 min after administration. Images on the right demonstrate fusion of the probe with vascular dextran marker. **E.** Intravital microscopy images of the distribution of control untargeted and scVEGF targeted fluorescent liposomes in the vasculature of MDA-MB-231 tumors 1.5 h after administration. High magnification images (bottom) demonstrate extravasation of the untargeted liposomes due to EPR effect (red arrow) whereas no extravasation was detected for the targeted liposomes presumably due to high-affinity binding to the endothelial cells via VEGFR2 receptors.

Conclusion: Passive targeting of liposomal formulation of SPIONs could be an optimal solution for MRI detection of breast tumors and for development of therapeutic liposomes for MRI-guided therapy.

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References: (1) Onuki Y, et al. *Biomaterials* 2010; 31(27): 7132. (2) Imoukhuede PI, et al. *Cancer Medicine* 2014; 3(2): 225.