MR imaging of cancer vasculature using VEGF receptor-targeted dual contrast labeled liposomes

Yoshinori Kato[1,2], Wenlian Zhu[1], Marina V Backer[3], Ronnie C Mease[1,4], Susanta K Sarkar[5], Joseph M Backer[3], and Dmitri Artemov[1,2]

[1]JHU ICMIC Program, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [2]Department of Oncology, The Sidney Kimmel Comprehensive Cancer Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [3]SibTech, Inc., Brookfield, Connecticut, United States, [4]Division of Neuroradiology, The Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, Maryland, United States, [5]Translational/Clinical Imaging, Sanofi Oncology, Cambridge, Massachusetts, United States

Target Audience: Scientists who are interested in image-guided drug delivery and therapy, specifically those who are involved in cancer imaging and therapy.

Purpose: The goals of this study are to develop bimodal liposomes for image-guided, targeted delivery of therapeutic and diagnostic agents to vascular endothelial growth factor (VEGF) receptors overexpressed in tumor vasculature, and to evaluate the feasibility of using them to detect vascular changes in response to anti-angiogenic combination therapy.

Methods: Liposomal MRI probe loaded with GdDTPA/superparamagnetic iron oxide (SPIO) nanoparticles were prepared by a standard lipid hydration method, followed by extrusion.[1] The surface decoration of liposomes with engineered single-chain version of VEGF (scVEGF), site-specifically derivatized with PEGylated lipid,[2] was performed by post-insertion method. The physicochemical properties of liposomes were characterized prior to *in vivo* experiments. Human breast cancer MDA-MB-231/luc cells that stably express luciferase[2] were orthotopically inoculated into the mammary fat pad of female athymic nude mice (1.2×10^6 cells/50 μL). All MRI studies were performed on a horizontal bore 9.4T Bruker Biospec scanner. Anesthetized animals were placed in the magnet with the tumor positioned in a custom-built, single-turn volume coil. Two-hundred microliters of the liposomal MRI probe were administered via tail vein injection, and the images were acquired up to 5 days post-injection. To determine the intratumoral distribution of the GdDTPA/SPIO dual labeled liposomes, T_2-weighted spin echo (TE/TR=21/4000 ms) and T_2*-weighted gradient echo (TE/TR=2/100 ms) images were acquired. The integrity of the liposomal probe was monitored using T_1 measurements as a decrease in T_1 is expected if GdDTPA is released and diffuses away from the confinement of the intact liposomes.[1,3] At the end-point, all tumors were removed and snap-frozen for immunohistochemistry to validate imaging results. Also, single-photon emission computed tomography (SPECT) imaging with scVEGF-PEG-DOTA/^{99m}Tc (scVEGF/^{99m}Tc tracer),[2] as well as a complementary *in vivo* optical imaging with scVEGF-decorated liposomes labeled with indocyanine green (ICG) were implemented.

Results: The diameters and zeta-potential of the control and scVEGF-decorated liposomes were 138 and 153 nm, and -11.6, and -11.2 mV, respectively. scVEGF-decorated liposomes contained 50-100 molecules of scVEGF per liposome. In a preliminary experiment, scVEGF-liposomes were effectively delivered and retained in the tumor as detected by T_2-weighted images (**Fig. 1**), presumably due to the active targeting of the liposomes to VEGFR. The liposomes remained stable within the tumor as no significant T_1 changes were observed (2.39 sec for Pre *vs.* 2.35 sec for 4-6 hrs). SPECT imaging of the tumor with scVEGF/^{99m}Tc tracer verified the overexpression of VEGFR in tumor vasculature.

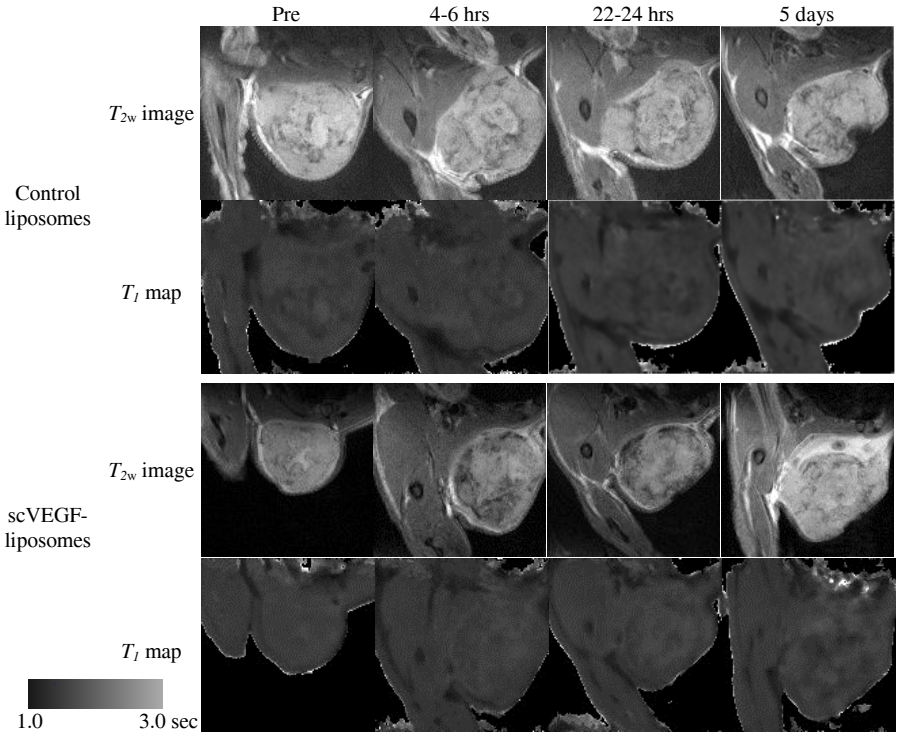

Fig. 1. MR images of orthotopic MDA-MB-231/luc xenografts following the intravenous administration of control or scVEGF-decorated liposomes loaded with GdDTPA and SPIO nanoparticles. The negative enhancement in T_2-weighted images represents the location of the liposomal probes, while decreases in T_1 values in T_1 map indicate the degradation of the liposomal probes because the released GdDTPA generates T_1 effect once it diffuses beyond the range of T_2/T_2* effect of SPIO particles that are relatively immobile due to their large size.[1, 2]

Discussion: scVEGF-decorated liposomes bind to VEGF receptors and selectively accumulate in tumor vasculature overexpressing these receptors, similar to other scVEGF-based contrast agents.[2] scVEGF-decorated liposomes are, most likely, eventually internalized by endothelial cells via VEGFR-mediated endocytosis, allowing for specific delivery of contrast and co-encapsulated therapeutic agents to VEGFR-2 overexpressing tumor endothelium. The advantages of the liposomal carrier over small molecular probes are i) simultaneous encapsulation of dual MRI probes and therapeutic agents for image-guided drug delivery; ii) increased MR sensitivity due to high loading of probes within a carrier; and iii) longer circulation time of the carrier which improves distribution to the target site. As expected, the scVEGF-mediated targeting resulted in improved delivery and retention of liposomes in the tumor. Since the control liposomes may also accumulate in the tumor due to the enhanced permeability and retention effect,[4] histological validation of the specific binding of scVEGF-liposomes to VEGF receptors in tumor endothelial cells by immunohistochemistry is necessary to verify targeted delivery, which is in progress. We are also exploring *in vivo* optical imaging with VEGFR-targeted, ICG-labeled liposomes to independently validate MRI results.

Conclusion: scVEGF-decorated liposomes loaded with a combination of MRI contrast agents and an anticancer agent can serve as a theranostic agent for image-guided, anti-angiogenic combination therapy that will provide for simultaneous monitoring of the delivery and drug release.

References: 1. Kato Y, et al. *Magn Reson Med* 61: 1059 (2009). 2. Backer MV, et al. *Nat Med* 13: 504 (2007). 3. Onuki Y, et al. *Biomaterials* 31: 7132 (2010). 4. Matsumura Y, et al. *Cancer Res* 46: 6387 (1986).