Lectinized Liposomes for Multimodal In Vivo Molecular Imaging of the Tumor Endothelium

A. P. Pathak[1], Y. Kato[1], and N. Benoit[1]

[1]JHU ICMIC Program, Russel H. Morgan Dept. of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, MD, United States

INTRODUCTION: Noninvasive imaging is proving to be indispensable for studying tumor angiogenesis [1]. Here we describe the development of *de novo* lectinized liposomes that improve our ability to image the structural and functional changes in tumor blood vessels during angiogenesis, using molecular magnetic resonance imaging (MRI). Contrast-enhanced, high-resolution, *in vivo* MR studies of tumor angiogenesis have been limited because conventional MR contrast agents have relatively short circulation half-lives, and extravasate from permeable tumor vessels. We have developed a ubiquitous, blood vessel-specific contrast agent targeted to a lectin from *Bandeiraea Simplicifolia* (GS-1), a plant-derived carbohydrate-binding protein that is non-immune in origin, and has been shown to bind to α-D-galactosyl residues expressed on endothelial cells [2]. Initial results from *in vitro*/*in vivo* MRI and optical imaging experiments demonstrate that multi-modal, targeted vascular endothelium-specific liposomes greatly enhance our ability to characterize tumor angiogenesis.

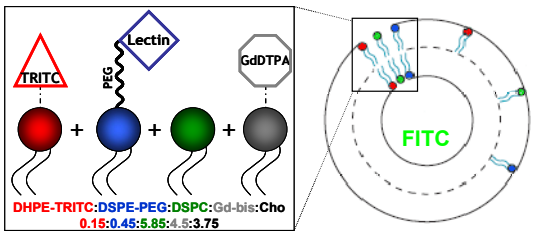

Fig.1: Inset shows the 4 phospholipids that constitute the liposome contrast agent. The first lipid incorporates Gd-DTPA, the second a fluorophore (TRITC) tag, and the third, polyethylene glycol (PEG) to achieve long circulation in vivo. The surface of this liposome is modified so that GS-1 lectin is attached to the PEG arm to yield our dual modality, targeted contrast agent.

METHODS: Dual contrast liposomes were synthesized from a blend of four phospholipids and cholesterol, using the ultrasonication method. **Fig.1** shows a cartoon illustrating the lipid blend employed, and the resulting "decorated" liposome**.** DSPE-TRITC phospholipid enables fluorescence detection of the liposomes (Ex=546 nm, Em=620 nm), while GdDTPA-bis permits MRI detection. Fluorescein was entrapped in liposomes and their size made uniform by extrusion. Following extrusion, excess fluorescein was removed by filtering with a Sephadex column. Liposome size was characterized using dynamic light scattering on a zeta sizer. Half the liposomes served as control liposomes while the other half underwent surface-modification with lectin (Lx) GS-1. EDC/Sulpho-NHS was used to activate carboxyl groups on the carboxylated DSPE-PEG for coupling with the amino group of the GS-1. Following synthesis, Lx- and control-liposomes were tested *in vitro* and *in vivo* as described below.

In Vitro Characterization: To test the labeling ability of Lx-liposomes we employed 2H11 cells, which are an excellent model of tumor endothelial cells because they have been shown to express all major tumor endothelial cell surface markers [3]. We employed MDA-MB-231 human breast cancer cells as controls. Cells were incubated with either Lx-liposomes, control-liposomes, free BSA-GdDTPA or cell culture medium for 8h, washed with medium and spun down to form pellets. Cell pellets were imaged on a 9.4T MR spectrometer using a saturation recovery method combined with SNAPSHOT FLASH imaging (flip angle=10°, TE=1.3ms). A 2mm thick coronal slice of the cell pellets was selected and a 128×64 matrix acquired, with 32×12mm^2 field of view for twelve relaxation delays (0.01, 0.02, 0.04, 0.08, 0.1, 0.2, 0.4, 0.8, 1.0, 2.0, 2.5, 3.0s). T1 maps were calculated using ImageJ. Following MRI, cell pellets underwent florescence imaging and mean fluorescence for each sample computed.

In Vivo Characterization: To test the ability of Lx-liposomes to label tumor vessels *in vivo*, we imaged two MDA-MB-231 human breast cancer xenograft bearing animals at 9.4T using a custom-built surface coil. Tumors were imaged prior to contrast administration and four hours after i.v. administration of Lx- or control-liposomes. MR images were acquired using a T1w, 3D FLASH angiography sequence (TE/TR=1.5/11ms), 12×12×12mm^3 FOV and 128×64×64 matrix with fat suppression.

RESULTS: *In Vitro Characterization*: **Fig. 2a** summarizes the results of the MRI-cell labeling experiment. One can clearly observe the shortened T1 of the 2H11 cells labeled with Lx-liposomes (1.7s) relative to the T1's (~2.0s) of the other samples. Quantification of the fluorescence of each cell pellet is summarized in **Fig. 2b** wherein one can see that the 2H11 cells labeled with the Lx-liposomes exhibited the highest fluorescence compared to the remainder of the samples. Background fluorescence from the 2H11 cells labeled with control-liposomes and MDA-MB-231 cells was also detectable. *In Vivo Characterization*: **Fig.3 a-b** show pre-contrast MIPs while **Fig3c-d** show post-contrast MIPs acquired 4h after i.v. administration of control- and Lx-liposomes, respectively. The enhancement in **Fig3c** is homogeneous compared to that in **Fig3d,** in which the vessels are rendered conspicuous (arrows) due to targeted enhancement by the Lx-liposomes.

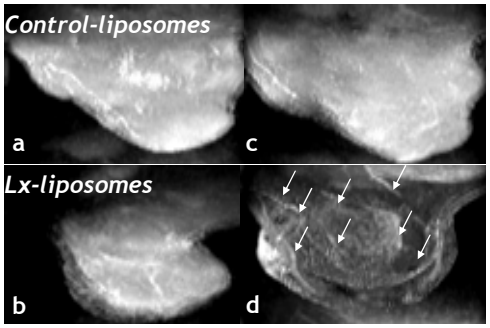

Fig.3: Maximum intensity projections from 3D, T1w MR images of: (a-b) MDA-MB-231 xenografts prior to liposome administration; 4h after i.v. injection of (c) control-liposomes, and (d) Lx-liposomes. Arrows in (d) indicate labeling of tumor vasculature.

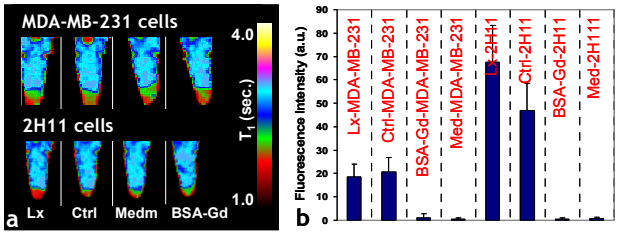

Fig.2: T1 maps of: 2H11 endothelial cells or MDA-MB-231 breast cancer cells incubated with Lx-liposomes, control-liposomes, medium and free BSA-GdDTPA. One can clearly observe shortened T1 of the 2H11 cells labeled with Lx-liposomes relative to T1's of other samples. Following MRI, mean red fluorescence of each pellet was computed (b). Although some background fluorescence from 2H11 cells labeled with control liposomes was observed, one can discern elevated fluorescence of 2H11 cells labeled with Lx-liposomes. MDA-MB-231 cells exhibited background fluorescence.

DISCUSSION: The *in vitro* data demonstrate the feasibility of dual contrast, i.e. MR and optical imaging of these (~140nm) lectinized liposomes. Under *in vitro* conditions, non-specific uptake of liposomes by cells does occur due to endocytosis. The *in vivo* data illustrate the ability of these targeted liposomes to enhance tumor vasculature for up to 4-6h, permitting high-resolution *in vivo* MRI and optical imaging of the tumor endothelium. For the range of lectin doses employed, we did not observe any *in vivo* toxicity from i.v. administration over a 24h period. We are currently working on encapsulating a therapeutic payload for targeted tumor delivery.

CONCLUSIONS: We have demonstrated for the first time the feasibility of synthesizing a novel multimodal contrast agent that is specifically targeted to murine tumor endothelial cells. This prototype lectin-decorated liposomal agent demonstrates the feasibility of dual modality i.e. MRI and optical imaging of tumor angiogenesis with widespread applicability, including *in vitro* assays, *in vivo* imaging and MR and optical microscopy.

REFERERENCES: **1.** McDonald DM, Choyke PL. *Nat Med.* 2003 Jun;9(6):713-25. **2.** Laitinen L. *Histochem J.* 1987 Apr;19(4):225-34. **3.** Walter-Yohrling J et al. *Clin Cancer Res.* 2004 Mar 15;10(6):2179-89.

ACKNOWLEDGEMENTS: Research supported by NIH R21CA128793-01.