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

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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.

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. Fluorescence was entrapped in liposomes and their size made uniform by extrusion. Following extrusion, excess fluorescence 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 128x64 matrix acquired, with 32x12mm³ 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 fluorescence 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 specific contrast agent targeted to a lectin from 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.

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 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.

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.


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