A New Lectin-targeted Contrast Agent for MR and Optical Molecular Imaging of Vascular Endothelium

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INTRODUCTION: Knowledge of the 3D architecture of tumor blood vessels is crucial from a tumor biology perspective [1] and for understanding the physiological underpinnings of image contrast in MRI [2]. While microscopy is powerful for dissecting molecular features of the vasculature, MRI and other methods make it possible to image vessel function in vivo [2]. Compared to the ~700nm resolution of microscopy, *in vivo* MRI has resolutions in ~100-500μm range. Our goal is to develop new imaging techniques to bridge this resolution gap for *in vivo* and *ex vivo* molecular MRI (MMRI) of tumor angiogenesis. Here we describe the development of a ubiquitous blood vessel-specific contrast agent targeted to a lectin from *Bandeiraea Simplicifolia* (GS-1), which is 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 [3]. Our initial results from *ex vivo* molecular MR microscopy (μMMRI) and optical microscopy of the murine vasculature, demonstrate that multi-modal, targeted vascular endothelium-specific contrast agents can greatly enhance our ability to image angiogenesis *ex vivo* and *in vivo* using MRI.

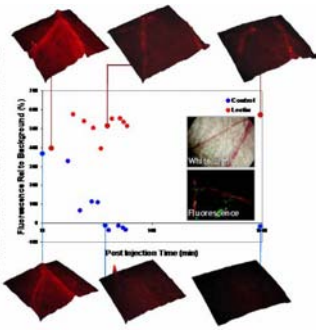

FIGURE 1 Plot of normalized fluorescence intensity versus post-injection time obtained from the ears of two different mice. The lower panel of fluorescence images is from the ear of a control mouse that only received an i.v. injection of red neutravidin microspheres and clearly shows the fluorescence plummeting to background levels 1h post-contrast, with no detectable fluorescence 18h later. In contrast, the upper panel of fluorescence images from the ear of a mouse that first received biotinylated-lectin followed by red neutravidin microspheres, clearly shows fluorescence that is visible 1h post-contrast and is sustainable 18h later. The upper inset shows an image of the vessels in the mouse ear under white light. The lower inset shows an immunofluorescence image of a mouse ear section from a mouse injected with the biotin-lectin/ neutravidin-microsphere combination, wherein one can clearly see punctate red microspheres attached to the vessel lumen and autofluorescing erythrocytes in the green channel.

METHODS: We conducted a series of experiments to determine the feasibility of targeting murine blood vessels using the lectin GS-1: *Expt. 1*: *To determine the feasibility of labeling and imaging murine blood vessels with lectin in vivo with optical imaging*: Two anesthetized female mice were used. The first was injected with 0.2ml of 2mg/ml solution of biotinylated lectin GS-1 via the tail vein. 40min later, both mice received 0.2ml (1:5 suspension) of 40nm Neutravidin-labeled red fluorescent microspheres i.v. 180min later, both mice received a second 0.2ml infusion of Neutravidin-labeled red fluorescent microspheres i.v. The ear of each animal was imaged every 5-7min, at 10× over one hour and then again the next day using an intravital fluorescence microscope. Regions were selected from vessels (I_v) and background (I_0) and the relative (%) fluorescence enhancement calculated as: $(I_v-I_0/I_0)\times100$.

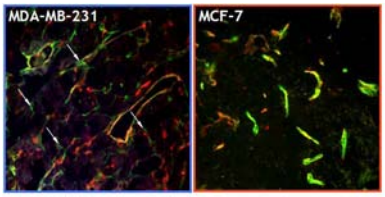

FIGURE 2 Immunofluorescence images of a tissue section from an MDA-MB-231 breast tumor xenograft showing BSA-FITC (green) and blood vessel endothelia stained with CD34 (red). One can clearly see extravasated BSA-FITC (arrows) outside the CD34 stained blood vessel lumina. In contrast, immunofluorescence images of a tissue section from an MCF-7 breast tumor xenograft showing lectin GS1-FITC (green) and blood vessel endothelia stained with CD34 (red) clearly show that lectin-FITC does not extravasate and labels the same endothelia as CD34, as is evident from the excellent colocalization of the two.

Expt. 2: *To determine if lectin-FITC binds to vessel lumen or leaks out in tumors*: For this, two anesthetized female mice were used. The first bore a MCF-7 tumor xenograft and was injected with 0.2ml of 1mg/ml solution of GS-1-FITC (114kDa) via the tail vein. The second bore a MDA-MB-231 xenograft and was injected with 0.2ml of 1mg/ml solution of BSA-FITC (70kDa) via the tail vein. 3h later, mice were sacrificed and 10μm tumor cryosections stained for endothelial cells using CD34 and images acquired on a fluorescence microscope with FITC/TRITC filters.

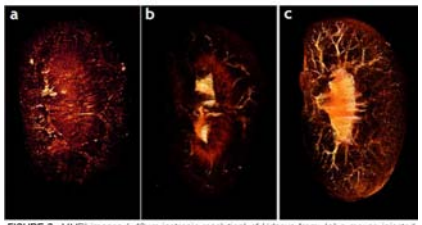

FIGURE 3 μMMRI images (~40μm isotropic resolution) of kidneys from: (a) a mouse injected with BSA-GdDTPA, (b) a mouse injected with BSA-GdDTPA followed by ligation of the renal artery before excision of the kidney, and (c) a mouse injected with the biotin-lectin+avidin+biotin-BSA-GdDTPA combination. One can clearly see a progressive improvement in MR contrast arising from the small vessels of the renal cortex from a-c.

Expt. 3: *To determine if lectin improves visibility of renal vessels in μMMRI?* Three anesthetized female mice were used. Mouse1 received 0.2ml of 60mg/ml solution of BSA-Gd-DTPA via the tail vein. Mouse2 received 0.2ml of 60mg/ml solution of BSA-Gd-DTPA via the tail vein and the renal artery was ligated before excising the kidney. Finally, Mouse3 received 0.3ml of 10mg/ml solution of biotinylated lectin GS-1 via the tail vein + 0.3ml of 10mg/ml of streptavidin via the tail vein 24h later + 0.2ml of biotin-albumin-GdDTPA (60mg/ml) i.v. 3h later. All animals were euthanized 60min post-contrast and kidneys excised and fixed. T1-weighted 3D spin echo images were acquired at a resolution of ~40×40×40μm on a 400MHz Bruker spectrometer equipped with microimaging gradients.

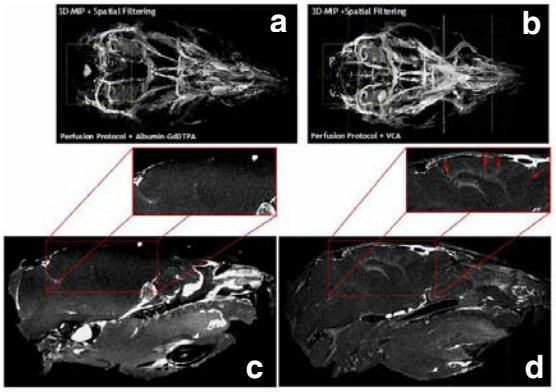

FIGURE 4 Maximum intensity projection μMMRI images (51×55×55 μm³ resolution) of heads from: (a) a mouse perfused with agarose doped with BSA-GdDTPA, and (b) a mouse injected with the biotin-lectin+avidin+biotin-BSA-GdDTPA combination followed by perfusion with the BSA-GdDTPA-doped agarose. (c) Only larger cortical vessels are visible (inset), while in (d) the smaller cortical vessels are also more conspicuous (inset).

Expt. 4: *To determine if lectin improves visibility of cortical vessels in μMMRI:* Two anesthetized female mice were used. Mouse1 was perfused with a 1% solution of type VII, low gelling temperature agarose doped with 33mg/ml of albumin-GdDTPA and 5mg/ml of BSA-FITC, followed by perfusion fixation with PFA. Mouse2 received 0.3ml of a 10mg/ml solution of biotinylated lectin GS-1, via the tail vein + 0.3ml of 10mg/ml of streptavidin via the tail vein 24h later + i.v. administration of 0.2ml of biotin-albumin-GdDTPA (60mg/ml) 3h later, followed by perfusion with the doped agarose and perfusion fixation. T1-weighted 3D spin echo images were acquired at a resolution of ~50×50×50μm on a 400MHz Bruker spectrometer equipped with microimaging gradients.

RESULTS: Upper panel in **Fig. 1** demonstrates the feasibility of long-term labeling of blood vessels in vivo with lectin. **Fig. 2** shows colocalization of i.v. administered lectin-FITC with the endothelial marker, CD34, i.e. successfully tagged MCF-7 tumor vessels. **Fig. 3** demonstrates the superior performance of the lectin targeted contrast agent compared to an intravascular contrast agent. **Fig. 4a** is an *ex vivo* μMRI of a mouse head prepared by perfusion fixation (PF) followed by perfusion with 1% agarose doped with BSA-Gd-DTPA, wherein no cortical vessels are distinguishable (inset). However, in **Fig. 4b**, an *ex vivo* μMMRI image of another mouse head prepared by i.v. administration of biotinylated lectin+avidin+biotinylated BSA-Gd-DTPA, followed by PF and perfusion with Gd-doped 1% agarose, one can clearly visualize cortical vessels (inset).

DISCUSSION/CONCLUSIONS: The preliminary data presented here demonstrate: (a) it is possible to label and image murine vessels in vivo using lectin, (b) optical analogs of the lectin-based contrast agent do bind to the vessel lumen without extravasating in tumor xenografts, (c) a three-step labeling protocol comprising of biotinylated-lectin, followed by a streptavidin linker, followed by biotinylated BSA-GdDTPA does improve visualization of renal and cortical vessels for *ex vivo* μMMRI. We are currently in the process of synthesizing and characterizing the pharmacokinetics of a one-step lectin contrast agent and have begun *in vivo* studies. These preliminary data indicate that a lectin-based contrast agent has the potential to yield a plethora of information regarding tumor angiogenesis using MRI and μMRI.

REFERERENCES: **1.** McDonald DM, Choyke PL. *Nat Med.* 2003 Jun;9(6):713-25. **2.** Pathak AP et al. *Methods Enzymol.* 2004;386:3-60. **3.** Laitinen L. *Histochem J.* 1987 Apr;19(4):225-34.