Towards the Detection of Hepatocellular Carcinoma with a pH-Responsive Dendritic MRI Contrast Agent

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Abstract: A new pH-responsive MRI contrast agent has been synthesized from a biotinylated G5-PAMAM dendrimer. The agent shows a substantial relaxivity improvement of 4.5 $mM^{-1}s^{-1}$ per gadolinium ion at 9.4T over the pH range 6.0 to 8.4. In vivo studies revealed that biotin-avidin-G5 Gd conjugate showed preferential uptake in liver tissues.

Introduction: Hepatocellular carcinoma (HCC) is the 4th largest cause of cancer mortality worldwide and the 8th leading cause in the United States.[1] Progress for HCC detection has been hampered by the diverse and heterogeneous nature of this cancerous pathology, which causes each patient's tumor to have unique attributes that may exhibit differential responses to detection. Perhaps most importantly, successful HCC treatment is highly dependent on the ability to diagnose patients at early stages of disease and to identify early response to therapy.[2] The microenvironment within tumors is significantly different from that in normal tissues. Extracellular pH in solid human tumors is often 0.4 units lower than in healthy tissues. Several promising Magnetic Resonance Imaging methods have been explored that detect differences in pH between normal and tumor tissues, but further improvements are required before translation to the clinic.[3] Here, we report a novel pH-responsive dendritic MRI agent with improved sensitivities and tissue targeting for the detection of HCC within in vivo animal models.

Methods: We have recently succeeded in biotinylating the surface of a G5-PAMAM dendrimer. The number of biotin molecules on the dendrimer's surface was determined using a commercially-available HABA-avidin assay, which revealed that an average of 3.1 molecules of biotin reside on the surface of a G5 dendrimer. A pH-responsive Gd-DOTA-4AmP analogue was conjugated to the amines on the surface of a biotonylated G5-PAMAM dendrimer via a benzyl-thiourea linkage that achieves excellent synthesis yields and purities for this polymer system. MR relaxivity measurements of the G5 dendritic conjugate were conducted at 1.0 mM and 20°C, using a 9.4T Bruker Biospin small animal MRI scanner. The pH of the sample was adjusted without changing final sample concentrations. The dendritic MRI contrast agent was used for in vivo MRI studies of Hsd:ICR(CD-1) mice. A solution of 40 μM/Kg Avi-Bt-G5-Gd conjugate was administered via tail vein to image liver in a live mouse. Axial images of the liver were obtained with a T1-weighted spin-echo sequence [TR/TE 600/8 ms, FOV 40x40 mm, image matrix 128x128, slice thickness 2mm]. The pre-injection image was subtracted from the post-injection image to highlight the contrast change created by the contrast agent.

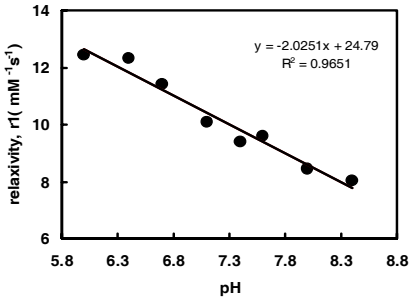

Figure 1. The pH-responsive relaxivity of the Bt-G5Gd dendrimer. MR relaxivity measurements were conducted at 1.0 mM, 20°C and 9.4T magnetic field strength.

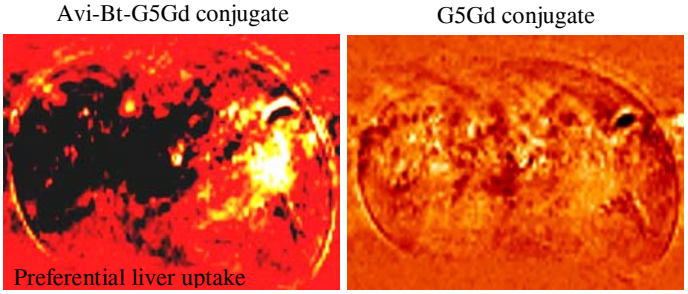

Figure 2. Approximately 40 μM/Kg of Avi-Bt-G5-Gd conjugate was injected via tail vein to image liver tissue in an in vivo mouse model. Axial images of the liver were obtained with a T1-weighted spin-echo sequence.

Result: The water proton relaxivity of the biotinylated G5-dendritic conjugate, $(N\{CS\}N\text{-}Bn\text{-}(Gd\text{-}DOTA\text{-}4AmP)_{96}\text{-}(biotin)_3$-G5-PAMAM dendrimer, increased from 8.0 to 12.5 $mM^{-1}s^{-1}$(on a per Gd basis) on changing the solution pH from 8.4 to 6.0 (9.4T, 20 ^{0}C; Figure 1). The change in relaxivity of 4.5 $mM^{-1}s^{-1}$ over this pH range is a tremendous improvement (at 9.4T) relative to the small molecule MRI contrast agent, which only shows a change in relaxivity of approximately 1.5 $mM^{-1}s^{-1}$ (0.5T). In addition, the overall relaxivity of the dendritic MRI contrast agent is 288 times greater than the relaxivity of the small molecule MRI contrast agent, which indicates a great sensitivity improvement for detecting the dendritic MRI contrast agent. The Avi-Bt-G5Gd conjugate shows hepatocellular uptake in a mouse model. However, injection of the non-biotinylated G5Gd dendrimer does not show accumulation of the agent in the liver.

Conclusion: The Bt-G5Gd dendritic MRI probe showed a substantial improvement in the change of relaxivity over the physiological pH range. The Avi-Bt-G5Gd dendritic MRI probe exhibited targeting to liver tissue. The combination of dendritic MRI probes that are pH-responsive and target liver tissue provides an excellent platform for detecting hepatocellular carcinoma within in vivo animal models.

References:

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