Development of Targeted Paramagnetic Nanoparticle for Non-Invasive Tumor Imaging

Meser M. Ali¹, Nadimpalli RS Verma¹, Janic Branislava¹, James R Ewing², Robert A Knight², and Ali S Arbab¹ ¹Radiology, Henry Ford Hospital, Detroit, Michigan, United States, ²Neurology, Henry Ford Hospital, Detroit, Michigan, United States

INTRODUCTION: The unique structural features of many solid tumors (hypervasculature, defective vascular architecture, and impaired lymphatic drainage) lead to relatively selective extravasation and retention of long-circulating nanocarriers. This phenomenon ("passive targeting") is essentially the working principle of most clinically viable targeting strategies based on nanocarriers. While the long-circulating nanocarriers significantly increased tumor localization of the payload, some limitations exist. First, the targeting effect is highly dependent on the degree of tumor vascularization, angiogenesis and high interstitial fluid pressures (IFPs, typically found in breast tumors). Therefore, the passive targeting approach may not be always effective in all tumors. Therefore, progress toward the effective clinical treatment of breast cancer has been hampered due to ineffective drug delivery, combined with an inability to image the true extent of drug delivery to the breast tumor. The development of tumor-targeted nanoparticles holds great promise for efficacious therapy with minimum side effects. A hallmark of the tumor microenvironment in malignant breast tumor is extracellular acidosis [1], which can be exploited for targeted delivery of drugs and imaging agents. Recently, the pH-selective insertion and folding of pHLIP (pH Low Insertion Peptide) in membranes demonstrated to target acidic tissue *in vivo*, including solid

tumors (human and mouse) [2]. Here, we demonstrate that pHLIP-tagged nanoparticles bind to and are internalized by breast cancer cells in vitro. Systemic delivery of the Gd-G5-pHLIP leads to accumulation of the nanoparticles in a flank mouse model of breast cancer that are detected by optical and MR imaging.

MATERIALS AND METHODS: We have synthesized pH-responsive MRI nanoprobe, phosphonate G5-(GdDOTA-4AmP) (Figure 1) by following our published synthetic method [3]. The MW of the conjugated G5 dendrimer was estimated at 71,303 g/mole by maldi-tof analysis. This corresponds to a G5-dendrime with an average of 51 chelated Gd^{3+} ions per dendrimer. Gd_{51} -G5 dendrimer was reacted with heterobifunctional cross-linker, succinimidyl-4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate (sulfo-SMCC) to form reactive maleimides and then maleimide- Gd_{51} -G5

denotrimer was coupled with C-terminus cysteine group of biotinylated Bt-pHLIP (AEQNPIYWARYADWLFTTPLLLLDLALLVDADEGTCG-pegBiotin) (New England Peptide, Gardner, MA, USA). The conjugate was purified by diafiltration (C-10) to produce final conjugate, Gd-G5-Bt-pHLIP (Fig.1). Biotin molecule is attached to the C-terminus of pHLIP in order to quantify the number of pHLIP peptides conjugated with Gd₅₁-G5. The HABA assay (Pierce Chemical) with biotin and avidin revealed that on average 3.1 molecules of biotin are present in Gd₅₁-G5-Bt-pHLIP dendrimer. Since biotin is attached with pHLIP peptide, therefore, 3.1 pHLIP peptides are also present in each Gd₅₁-G5 particle. Finally, Rhodamine dye was conjugated to amines surface of preloaded Gd₅₁-G5-Bt-pHLIP₃ in order to achieve final conjugate Rhodamine-Gd₅₁-G5-Bt-pHLIP₃ as shown in Fig. 1. Unreacted dye was removed by diafiltration (using C-10). A T1WI

Look-Locker [4] sequence (TR/TE=2000ms/2.2ms, 24 echoes, 128x64 matrix, 34x34 mm² FOV, 5 slices, 2.0 mm thick) was run serially for roughly 105 minutes to produce estimates of T_1 at approximately 2.5 minute intervals.

DISCUSSION: The size of Bt-pHLIP conjugated dendrimer, Gd-G5 (Fig. 1) is estimated by transmission electron microscopy and the particle size is approximately 7.5 \pm 0.6 nm. To study pH-dependent translocation of molecules across the cell membrane,







we have added Rho-Gd-G5-Bt-pHLIP to the cells and incubated for 3 h at pH 7.4 and 6.5. Then we have washed the cells at pH 7.4 to remove any reversibly bound peptide. The cellular uptake of Rho-Gd-G5-pHLIP was significantly higher at pH 6.5, as clearly demonstrated in Fig. 2. When Rho-Gd-G5 was used, the cellular uptake was considerable lower at both, pH 6.5 and 7.4 (data not shown). Therefore, non targeted fluorescent labelled nanoparticle, Rho-Gd-G5 did not exhibit any non-specific cellular uptake. Hence, we have shown the ability of pHLIP peptide for intracellular delivery of Gd-G5 nanoparticles in vitro at pH 6.5 but the same ability is attenuated significantly at neutral pH. We have created a mouse model of aggressive MDA-MB-231 breast cancer. The pharmacokinetics of Gd_{51} -G5-pHLIP was visualized in the MDA-MB-231 tumor over the course of 105 min post-contrast administration. These data sets were then analyzed using the Patlak graphical method to analyze the Kety equation on a pixel-by-pixel basis, relative to a T1 MR parametric map that was acquired prior to the injection of paramagnetic nanoparticle. T1-maps are generated at different time points after post contrast administration. T1-maps clearly demonstrate accumulation of the agent concentration within tumor over time.

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