Potential Application of Hyaluronic Acid in MR Imaging

W. Zhu[1], and D. Artemov[1]

[1]Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Introduction. The up-regulation of CD44 in breast cancer cells is generally correlated with poor prognosis[1]. CD44 also has been identified as a putative breast cancer stem-like cell marker[2]. Therefore, CD44 is an important breast cancer therapeutic target. We hypothesized that as a major CD44 ligand, hyaluronic acid (hyaluronan or HA) can be used as a carrier for MRI probes such as gadolinium, offering a simple and direct approach to access the CD44 status of breast cancer cells. HA-gadolinium conjugate may be also used as a blood pool contrast agent since HA is a highly water soluble and nonimmunogenic polysaccharide with molecular weight ranged from 5 to 20,000 kD.

Materials and Methods. HA (MW = 35 kD) was conjugated to BODIPY through a carboxylic reactive BODIPY® FL hydrazide. To produce an MRI agent, HA was reacted with ethylenediamine (EDA) at the carboxyl groups to produce a linker to conjugate to diethylenetriaminepentaacetic acid (DTPA). HA-EDA-DTPA was chelated to gadolinium by reacting with gadolinium chloride. The final product was purified by ultra-filtration. MDA-MB-231 cells were treated with 60 µg/ml HA-BODIPY for different length of time and observed with fluorescence microscope. For *in-vivo* mouse MRA studies, images were acquired with a 3D FLASH sequence (TE/TR = 2.5/8ms, flip angle 25 degree, FOV 48x28x28 mm, matrix size 256x80x80, NA = 8). HA-EDA-DTPA-Gd was injected through an intravenous catheter at the dose of 200mg/kg.

Results and Discussions. About 85% of MDA-MB-231 cells are CD44 positive[3]. We found that MDA-MB-231 cells showed strong binding to HA-BODIPY at 1hour, 2 hour, and 3 hour intervals as shown in Figure 1. No specific binding was observed in CD44 negative MCF-7 breast cancer cells[3]. The gadolinium content of our HA-EDA-DTPA-Gd as determined by ICP-MS was 13.9%, which corresponded to about 90% of Gd conjugation rate at the HA carboxyl sites. HA-Gd conjugate displayed a favorable blood clearance profile with extended circulation time, Figure 2. The images showed that HA-Gd is mainly cleared through kidney and bladder with no observable accumulation in other organs.

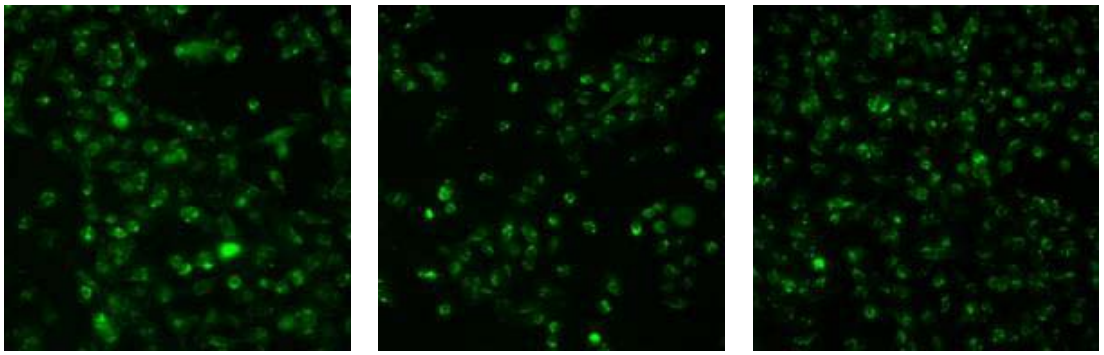

Figure 1. Binding of HA-BODIPY to MDA-MB-231 cells at the concentration 60 µg/ml. Fluorescence microscope images shown here were 1 hour (left), 2 hours (middle), and 3 hours (right) after the addition of HA-BODIPY.

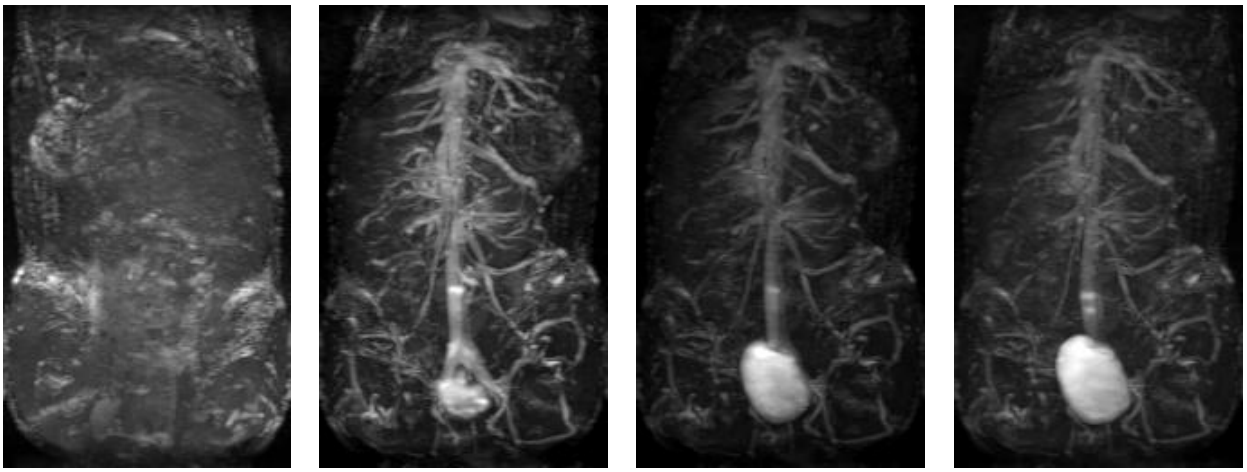

Figure 2. Maximum intensity projection of 3D FLASH images of a mouse injected with 200mg/kg HA-Gd. Pre-contrast, 30second, 9minutes, and 20 minutes post-contrast images are shown from left to right.

Conclusion. Preliminary data demonstrated the potential of HA as a targeting moiety to CD44 positive cancer cells. HA-EDA-DTPA-Gd conjugate also displayed a favorable blood clearance profile with extended circulation time. As such, the highly water soluble, non-toxic, biocompatible, and non-immunogenic HA offered great advantage as a blood pool contrast agent.

References. 1. Gotte, M. & Yip, G.W. Heparanase, hyaluronan, and CD44 in cancers: a breast carcinoma perspective. *Cancer Res* 66, 10233-7. 2. Reya, T., Morrison, S.J., Clarke, M.F. & Weissman, I.L. Stem cells, cancer, and cancer stem cells. *Nature* 414, 105-11. 3. Sheridan, C. et al. CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. *Breast Cancer Res* 8, R59 (2006).

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