Potential Application of Hyaluronic Acid in MR Imaging

W. Zhu\textsuperscript{1}, and D. Artemov\textsuperscript{1}

\textsuperscript{1}Russell H. Morgan Department of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, United States

\section*{Introduction.} The up-regulation of CD44 in breast cancer cells is generally correlated with poor prognosis\textsuperscript{1}. CD44 also has been identified as a putative breast cancer stem-like cell marker\textsuperscript{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 also be used as a blood pool contrast agent since HA is a highly water-soluble and non-immunogenic polysaccharide with molecular weight ranging from 5 to 20,000 kD.

\section*{Materials and Methods.} HA (MW = 35 kD) was conjugated to BODIPY through a carboxylic reactive BODIPY\textsuperscript{®} FL hydrazide. To produce an MRI agent, HA was reacted with ethylenediamine (EDA) at the carboxyl groups to produce a linker to conjugate to diethylenetriaminepentaaetic acid (DTPA). HA-EDA-DTPA was chelated to gadolinium by reacting with gadolinium chloride. The final product was purified by ultrafiltration.

MDA-MB-231 cells were treated with 60 µg/ml HA-BODIPY for different length of time and observed with fluorescence microscope. For \textit{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.

\section*{Results and Discussions.} About 85% of MDA-MB-231 cells are CD44 positive\textsuperscript{3}. We found that MDA-MB-231 cells showed strong binding to HA-BODIPY at 1 hour, 2 hour, and 3 hour intervals as shown in Figure 1. No specific binding was observed in CD44 negative MCF-7 breast cancer cells\textsuperscript{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.

\section*{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.


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