A novel Hyaluronan based contrast agent for non-invasive detection of Hyaluronidase by MRI

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Introduction The tumor microenvironment, including the interaction between tumor cells and stromal cells, and the extracellular matrix (ECM), is extremely important in tumor progression, aggressiveness and response to treatment. One of the most common ECM components is hyaluronan (HA). High molecular weight hyaluronan is antiangiogenic while its low MW breakdown products are proangiogenic and affect cell proliferation and migration leading to tumor metastasis. Hyaluronidase (HYAL) degrades high molecular weight HA into low molecular weight fragments thus altering the ECM to become permissive to angiogenesis.[1] Our goal is to detect HYAL non-invasively by MRI in order to predict the tilt of angiogenic balance within the tumor microenvironment. We have earlier proposed HA-GdDTPA-agarose beads as a smart probe for detection of HYAL activity by MRI.[2] Our new approach is to use recently suggested self-assembled hyaluronan nanoparticles[3] for shielding GdDTPA from the bulk water. This novel contrast agent will remain MRI silent until it reaches the tumor site and its hyaluronan top layer is degraded by HYAL allowing GdDTPA to contact with surrounding water molecules. **Methods** HA-GdDTPA was synthesized by conversion of hydrophilic HA into amphiphilic one by conjugating it with hydrophobic cholanic acid (CA), followed by binding of GdDTPA. Gadolinium content of the final product was detected by ICP-MS and revealed 2.5% Gd in 1 mg HA-GdDTPA. The conjugate was sonicated to form nanoparticles, which were characterized in terms of size using Light Spectroscopy and morphology using Transmission electron microscopy (TEM). Human epithelial ovarian carcinoma ES-2 cells were cultured in DMEM medium and used to test the ability to detect secreted HYAL by MRI after incubation with 0.5, 1.0, 2.0 and 4.0 mg/ml HA-GdDTPA nanoparticles. We compared R_2 relaxation rates in the medium incubated over night with cells and fresh medium after addition of different concentrations of HA-GdDTPA. The nanoparticles were prepared in DMEM medium, sonicated and added to the cells for 15 min. R_2 MRI measurements were acquired from a single coronal slice within the medium above the cells at Bruker Biospec 9.4 T scanner using Multi-slice multi-echo (MSME) pulse sequence with following parameters: nEchoes=60, TE/TR=8/5000 ms, FOV=2.81x2.81 cm, thickness=0.8 mm, NA=2, 256x256). For R_1 measurements MSME acquired from a single coronal slice at 12 TRs: 5000, 2000, 1000, 800, 700, 600, 500, 400, 300, 200, 100 and 50 ms with TE=8 ms, thickness=0.8 mm, 128x128). R_1 and R_2 maps were reconstructed on a pixel-wise basis. Relaxivities were calculated as the change in R_1 and R_2 as a function of Gd content. **Results and Discussion** HA-GdDTPA, a novel contrast agent for non-invasive HYAL detection, was synthesized and characterized using Light scattering, TEM and MRI. The nanoparticles have spherical shape with size of 200-250 nm and remain stable during 7 days at physiological conditions. We demonstrate here how r_1 and r_2 relaxivities altered in response to degradation of HA-GdDTPA nanoparticles by hyaluronidase secreted by ES-2 ovarian carcinoma cells.

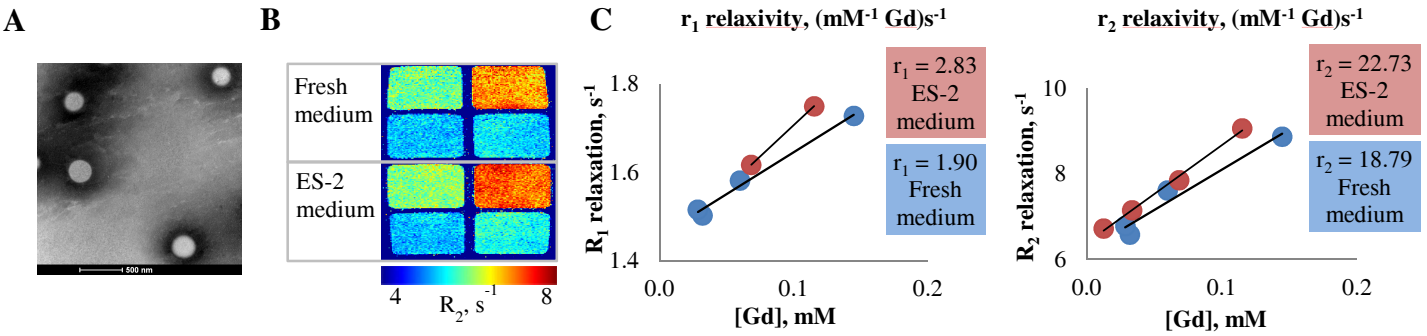

Figure. (A) TEM image of 1mg/ml HA-GdDTPA nanoparticles dissolved in PBS and sonicated for 2 min. The TEM sample was stained with Vanadate stain. (B) Comparison of R_2 relaxation maps of cells with overnight (ES-2) and fresh medium after addition of 0.5, 1.0, 2.0, 4.0 mg/ml of HA-GdDTPA nanoparticles. (C) Comparison of r_1 and r_2 relaxivities between ES-2 and fresh medium.

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2. Shiftan L. et al. *Cancer Res*. 65, 2005.
3. Choi K. Y. et al. *Biomaterials*. 31, 2010.