Synthesis and evaluation of CREKA-Tris(Gd-DOTA)3 for MR molecular imaging of breast cancer

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Purpose: MRI is a powerful medical imaging modality to display anatomical structures of body, especially useful for the detection and characterization of diseased soft tissues such as solid tumors. Various targeted contrast agents have been prepared for cancer molecular imaging with MRI. However, MRI is not effective for molecular imaging because of its low sensitivity. Most of these agents could not generate sufficient contrast enhancement because of low concentration of biomarkers on cancer cell surface. CREKA is a tumor-homing pentapeptide (Cys-Arg-Glu-Lys-Ala) specifically homes to tumors by binding to fibrin and fibronectin associated plasma protein clots in tumor stroma. Thus, we synthesized and evaluated a new tumor-targeted contrast agent CREKA-Tris(Gd-DOTA)$_3$ for MR molecular imaging of breast cancer.

Methods: CREKA-Tris(Gd-DOTA)$_3$ was synthesized by conjugating CREKA with three Gd-DOTA monoamide chelates via a maleimide-functional trialkyne scaffold by thiol-maleimide and azide-alkyne click chemistry, respectively. Mice bearing 4T1-GFP-Luc2 breast tumors in fat pad were studied at 2-3 weeks post tumor implant. For peptide binding study, mice were sacrificed and tumor tissue was collected and sectioned after 4 h post injection with CREKA-Cy5 or a non-targeted peptide CERAK-Cy5. Tumor slices were stained with rabbit polyclonal anti-mouse fibronectin antibody (abcam®), followed by rhodamine red conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc.). The MRI study was performed using a Bruker Biospec 7 T MRI scanner (Bruker Corp., Billerica, MA, USA) with a volume RF coil. Mice were anesthetized with a 2% isoflurane-oxygen mixture in an isoflurane induction chamber. Mice were injected with Prohance™, CREKA-Tris(Gd-DOTA)$_3$ or the non-targeted contrast agent CERAK-Tris(Gd-DOTA)$_3$ at a dose of 0.1 mmol-Gd^{3+}/kg, respectively. Fat suppression T_1-weighted 2D axial images were then acquired at different time points after the injection for up to 30 min.

Results and Discussion: The fibronectin immunostain of tumor slice was shown in Fig.1. Fluorescence from intravenously injected CREKA peptide (Fig. 1D) and fibronectin staining (Fig. 1C) colocalized (Fig.1E) in tumor sections of 4T1-GFP-Luc2 breast cancer xenografts. Little fluorescence was detected in the healthy tissue or the tumor from the mice injected with nontargeted peptide. These results indicate specific binding of CREKA to fibronectin in tumor tissue.

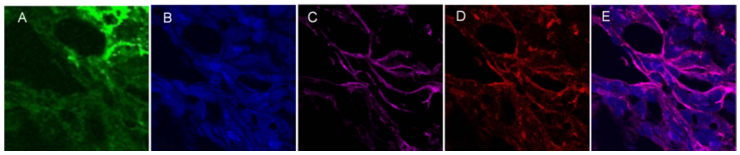

Fig.1. Tumor slices imaged by confocal microscope. A, GFP of tumor cells; B, cell nuclei stained by DAPI; C, Fibronectin stained by rhodamine red; D, CREKA-Cy5; E, Overlay of B, C and D.

The target contrast agent CREKA-Tris(Gd-DOTA)$_3$ showed much stronger tumor enhancement than ProHance™ and the nontargeted agent (Fig.2 B). CREKA peptide was able to deliver a sufficient amount of Gd-DOTA chelates to its molecular target for effective tumor molecular imaging with MRI. MRI can be effective for cancer molecular imaging if suitable molecular targets are identified.

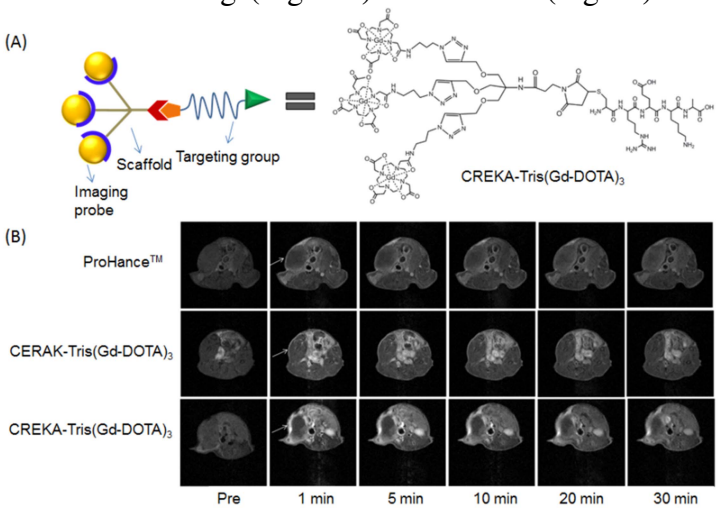

Figure 2. The structure of CREKA-Tris(Gd-DOTA)$_3$ (A) and the representative 2D axial T_1-weighted MR images of mice bearing a 4T1 breast orthotopic tumor before (pre) and at 1, 5, 10, 20, 30 minutes after the injection of Prohance™, CERAK-Tris(Gd-DOTA)$_3$ and CREKA-Tris(Gd-DOTA)$_3$ at 0.1 mM-Gd/kg. White arrows point to tumor. (B)