

MR Molecular Imaging of Breast Cancer Metastases with Peptide Targeted Tripod Macrocylic Gd(III) Chelates

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Purpose: MRI is a powerful medical imaging modality for the detection and characterization of diseased soft tissues such as solid tumors. MR molecular imaging has a great potential for detection and characterization of metastatic breast cancer if a suitable molecular target can be identified. However, currently available targeted contrast agents could not generate sufficient contrast enhancement for molecular MRI of the biomarkers on cancer cell surface due to low concentration of the biomarkers and low sensitivity of MRI. The extracellular matrix of malignant tumors has abundant accumulation of fibrin-fibronectin complexes that can be used as a suitable biomarker for effective molecular MRI of small breast cancer metastases. CREKA is a tumor-homing pentapeptide (Cys-Arg-Glu-Lys-Ala) that specifically binds to fibrin and fibronectin associated plasma protein clots in tumor stroma. Here, we synthesized and evaluated a tumor-targeted contrast agent CREKA-Tris(Gd-DOTA)₃ for MR molecular imaging of breast cancer metastases.

Methods: CREKA-Tris(Gd-DOTA)₃ was synthesized as previously reported. Seven- to eight-week-old female BALB/c mice were anesthetized with 2–3% isoflurane in O₂ and injected in the left ventricle of the heart with 1 × 10⁵ 4T1-GFP-Luc2 (metastatic murine mammary carcinoma) cells in 100 μL PBS. Tumor metastases were monitored by bioluminescence imaging. Mice bearing 4T1-GFP-Luc2 metastatic tumors were studied at 2-3 weeks post-injection. For peptide binding study, mice were sacrificed and tissues with metastases were collected and sectioned after 4 h post-injection with CREKA-PEG-Cy5 or the non-targeted peptide CERAK-PEG-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.). MRI study was performed using a Bruker Biospec 7 T MRI scanner (Bruker Corp., Billerica, MA, USA) with a volume RF coil. Mice were injected with CREKA-Tris(Gd-DOTA)₃ or the non-targeted contrast agent CERAK-Tris(Gd-DOTA)₃ at a dose of 0.15 mmol-Gd³⁺/kg, respectively. Fat suppression T1-weighted 3D FLASH sequence images were then acquired after the injection for up to 30 min. Mice were sacrificed and a high-resolution 3D FLASH sequence images was acquired. Mice were then imbedded in O.C.T and frozen in liquid nitrogen. Mice were sectioned and cryo-imaged. The 3D MRI and cryo-images were co-registered. Tumors detected by MRI and cryo-imaging were analyzed.

Results and discussions: Fig.1 shows the specific binding Cy5.0-PEG-CREKA to metastatic tumors in the liver. Strong red fluorescence was shown in metastatic tumors from the mice injected with Cy5.0-PEG-CREKA, whereas little fluorescence was detected in the metastatic tumors from the mice injected with the control probe. The fibronectin

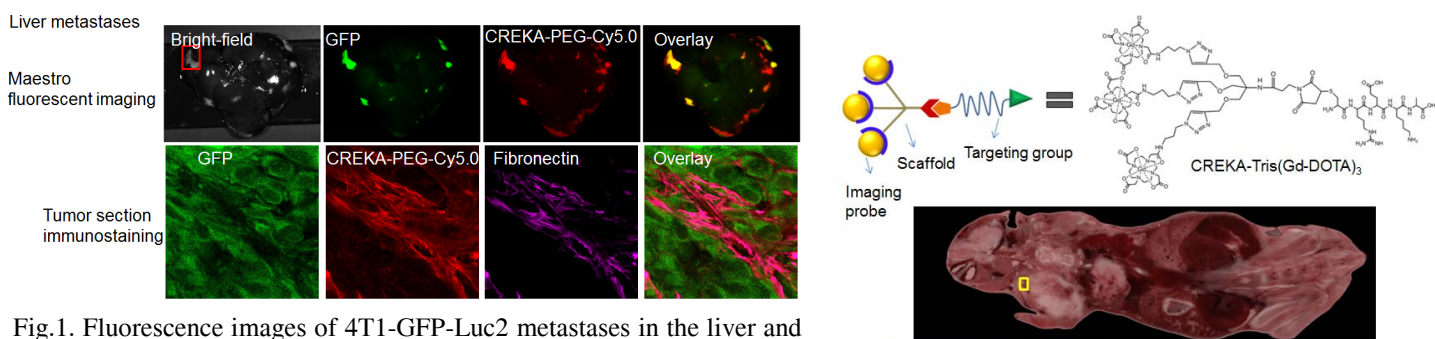


Fig.1. Fluorescence images of 4T1-GFP-Luc2 metastases in the liver and immunostaining of slice of the tumor metastases.

immunostaining of tumor slice indicates specific binding of CREKA to fibronectin in tumor tissue. CREKA-Tris(Gd-DOTA)₃ produced sufficient contrast enhancement of the metastatic tumors in different tissue. The co-registered MRI and cryo-images validated the results.

Conclusion: Molecular MRI with CREKA-Tris(Gd-DOTA)₃ is promising for effective detection of metastatic tumors of breast cancer.

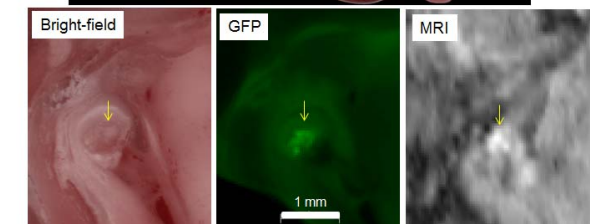


Fig.2. The structure of CREKA-Tris(Gd-DOTA)₃ and an example of molecular MRI of breast cancer metastases in bone marrow as validated by cryo-imaging.