Molecular MR imaging of micrometastasis of breast cancer

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Purpose: Metastatic disease is the primary cause of death in breast cancer patients. Early and accurate detection of breast cancer micrometastases is critical for improved prognosis and treatment. MR molecular imaging has a great potential for detection and characterization of breast cancer micrometastasis if a suitable molecular target can be identified. Currently available clinical contrast agents cannot generate sufficient contrast enhancement for small sized tumors. The extracellular matrix of malignant tumors has an abundant accumulation of fibrin-fibronectin complexes. CREKA is a tumor-homing pentapeptide (Cys-Arg-Glu-Lys-Ala) that specifically binds to fibrin-fibronectin clots in the tumor ECM.[1] Specific binding of a CREKA targeted contrast agent to fibrin-fibronectin complexes could generate sufficient enhancement for effective molecular MRI of breast cancer micrometastases. Here, we evaluated a targeted contrast agent CREKA-Tris(Gd-DOTA)$_3$ for MR molecular imaging of breast cancer micrometastases.

Methods: Female BALB/c mice (7-8 weeks old) were anesthetized with 2–3% isoflurane in O_2 and injected in the left ventricle of the heart with 1×10^5 4T1-GFP-Luc2 cells in 100 μL PBS. Tumor metastases were monitored by bioluminescence imaging for two weeks. The mice were injected with CREKA-PEG-Cy5 or the non-targeted peptide CERAK-PEG-Cy5. At four hours post injection, the mice were sacrificed and the metastatic tissues were collected and sectioned. 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 studies were performed using a Bruker Biospec 7 T MRI scanner (Bruker Corp., Billerica, MA, USA) with a volume RF coil. The mice were injected with CREKA-Tris(Gd-DOTA)$_3$[2] or the non-targeted contrast agent CERAK-Tris(Gd-DOTA)$_3$ at a dose of 0.15 mmol-Gd^{3+}/kg, respectively. Fat suppression T1-weighted 3D FLASH sequence images were then acquired after the injection for up to 30 min. Afterwards, the mice were sacrificed and high-resolution 3D FLASH sequence images were acquired. The mice were then imbedded in O.C.T, frozen in liquid nitrogen, and subsequently sectioned and cryo-imaged. The 3D MRI and cryo-images were co-registered and analyzed.

Results and Discussions: Fig. 1 shows the specific binding of CREKA-PEG-Cy5.0 to metastatic tumors in the lung. Strong red fluorescence was shown in metastatic tumors from the mice injected with CREKA-PEG-Cy5.0, whereas little fluorescence was detected in the metastatic tumors from the mice injected with the control probe. The fibronectin 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 tissues. The co-registered MRI and cryo-fluorescence images validated these results.

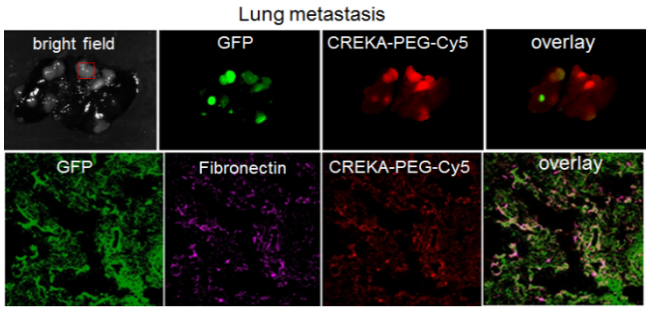

Fig.1. Fluorescence images of 4T1-GFP-Luc2 metastases in the lung (top) and immunostaining of slice of the tumor metastases (bottom).

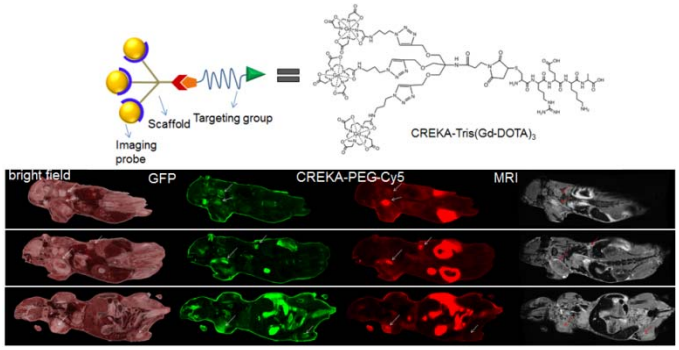

Fig.2. The structure of CREKA-Tris(Gd-DOTA)$_3$ and an example of image co-registration of molecular MRI and cryo-imaging of micrometastasis.

Conclusion: Utilizing CREKA-Tris(Gd-DOTA)$_3$ for molecular MRI is a promising technique for the detection of breast cancer micrometastasis.

Reference: 1) Simberg D, Duza T, Park JH, et al. *Proc. Natl. Acad. Sci. USA*. 104(3), 932-936 (2007). 2) Zhou Z, Wu X, Kresak A, Griswold M, Lu ZR. *Biomaterials*. 34(31), 7683-7693 (2013).