Evaluation of a Targeted Nanoglobular Gd Chelate for MRI Molecular Imaging of Prostate Tumor in an Orthotopic Mouse Model

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Introduction:

Magnetic resonance (MR) molecular imaging highly depends on the effectiveness of targeted contrast agents and the abundance of the molecular targets. A cyclic CLT1 peptide (CGLIIQKNEC) has been reported that it can recognize tumors by binding to fibrin-fibronectin complexes in tumor tissue [1]. The CLT1 peptide may be useful as a targeting moiety for MR molecular imaging of oncofetal fibronectin, a subtype of fibronectin only expression in tumor tissue to promote angiogenesis and tumor proliferation. Peptide CLT1-targted nanoglobular Gd(III) contrast agents in our previous work demonstrated significant enhancement in subcutaneous tumor models [2]. In order to better understand the effectiveness of the targeted agents, an orthotopic tumor model is necessary to simulate clinical cancers. The microenvironment surrounding tumor cells has important influence on the tumor's in vivo molecular imaging. Therefore, orthotopic tumor models are more valuable in evaluating targeted contrast agents for MR molecular imaging than the subcutaneous ones [3]. In this work, tumor accumulation of peptide CLT1 was examined with fluorescence imaging. The CLT1-targted nanoglobular contrast agent was investigated in male mice bearing prostate xenograft tumor with corresponding non-specific peptide (KAREC) conjugated agent as control.

Methods:

Cyanine 5 labeled probes and Contrast agents

Fluorescent probes, cyanine 5 labeled CLT1 and its control peptide KAREC, were prepared by reacting peptides with cy5 via a short PEG linker, 12-amino-4,7,10-trioxadodecanoic acid. The fluorescent probes were purified with HPLC and characterized by MALDI-TOF mass spectrometry. Peptide CLT1 targeted nanoglobular contrast agent was synthesized as described previously [2]. Similarly, control peptide KAREC conjugated nanoglobular contrast agent was synthesized following the similar procedure. Peptide KAREC has been reported to show no binding to fibronectin-fibrin complexes in tumors [1].

Fluorescence Imaging

To verify whether the CLT1 peptide can recognize the tumor in vivo, male mice bearing orthotopic PC-3 prostate xenograft tumors were intravenously injected with cyanine 5 labeled CLT1 and the control peptide KAREC. After 2 hours injection, the tumors and various organs were imaged with Maestro fluorescence system. Spectral fluorescence images were obtained using the appropriate filters for cy5 (excitation: 576-621 nm; emission: 635 nm long-pass filter; acquisition settings: 630-800 in 10 nm steps).

In vivo MR imaging

MR imaging was performed by using a 7 T Bruker Biospec small animal MRI system. Male nu/nu athymic mice ($N = 4$) bearing orthotopic PC-3 prostate tumors were injected with the contrast agents via a tail vein at a dose of 0.03 mmol-Gd/kg. A T1-weighted spin echo acquisition (repetition time/echo time = 500 ms /14 ms) was used to acquire the MR axial images. Signal intensity in the tumor tissue was measured and tumor contrast enhancement was expressed as signal-to-noise ratios (SNR).

Results and discussion:

The CLT1-Cy5 conjugate resulted in strong fluorescence in tumor tissue, whereas much less fluorescence was detected in tumor of the mouse injected with control peptide-cy5 (Figure 1). No fluorescence in healthy organs was detected for the mice injected with the CLT1-cy5 conjugate. The r_1 and r_2 relaxivities of the CLT1 targeted agent were 11.63 and 15.73 $mM^{-1}s^{-1}$ per Gd(III) chelate, and 11.44 and 15.37 $mM^{-1}s^{-1}$ for the control peptide conjugated agent at 1.5T. *In vivo* MRI showed that the CLT1 targeted agent resulted in much stronger contrast enhancement in the tumor tissue than the non-specific peptide control agent (Figure 2). Quantitative analysis of the SNR in the tumor tissue revealed that the CLT1-targeted contrast agent resulted in a higher SNR within tumor than the control.

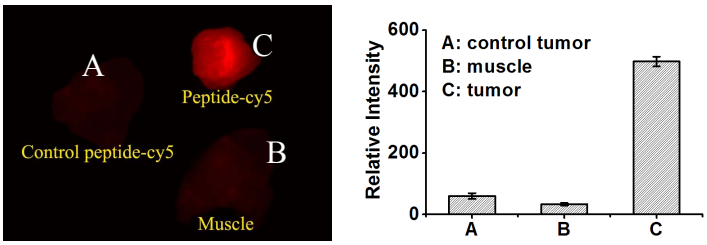

Figure1. Mice bearing orthotopic PC-3 prostate xenograft tumors were intravenously injected with 10 nmol of cyanine 5 labeled CLT1 and control peptide (KAREC). After 2h, the mice were sacrificed and the tumors and muscle were imaged with (Left) Cyanine 5 fluorescence image of control tumor (A), muscle (B) and tumor (C). (Right) Comparison of cy5 fluorescence intensity of tumors, control tumor and muscle. Tumor tissue injected with CLT1-cy5 showed strong fluorescence than that injected with control peptide conjugated cy5 and muscle.

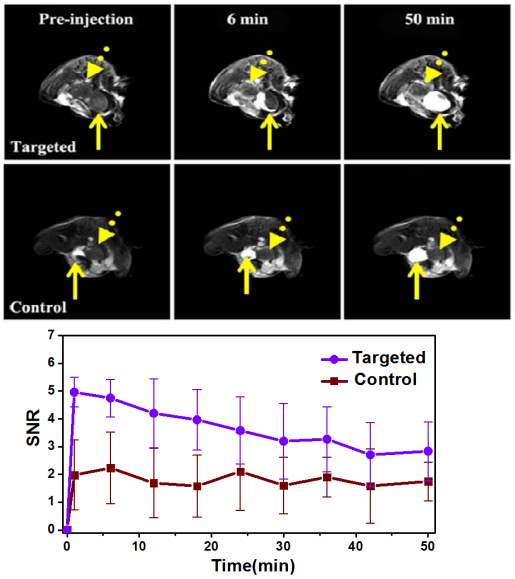

Figure 2. (Upper) 2D axial MR images of orthotopic prostate xenograft before, 6 min and 50 min after the administration of CLT1-targeted agent (targeted) and control peptide KAREC conjugated agent (control). Dashed arrows point tumors, solid arrows show bladder. (Lower) SNR in the tumor with targeted and control agent administrated at 0.03 mmol-Gd/kg in the tumor bearing mice.

Conclusions:

The CLT1-cy5 conjugate showed much higher accumulation in tumor than its control peptide in fluorescence imaging. Little fluorescence was detected in healthy organs with the peptide Cy5 conjugates. The CLT1-targeted nanoglobular contrast agent resulted in significant contrast enhancement in tumor for MR molecular imaging of oncofetal fibronectin at a relatively low dose as compared to the control agent. The results have demonstrated that the CLT1-targted nanoglobular contrast agent is effective for in vivo MR molecular imaging in an orthotopic mouse tumor model.

References:

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