Synthesis and Characterization cRGD-PEG Iron Oxide Nanoparticles of Noninvasive Imaging for Targeting $\alpha_v\beta_3$Integrins by MRI

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Abstract

We have synthesized a new and stable MR contrast agent, SPIO-mPEG-cRGD, that exhibits superior binding to the biologic target $\alpha_v\beta_3$ integrins. Selective targeting of $\alpha_v\beta_3$ integrins was achieved by conjugating cyclic RGD peptides to the superparamagnetic iron oxide nanoparticles (SPIO) surface, while poly(ethylene glycol) (PEG) were used for nonimmunogenic, nonantigenic and protein resistant. The signal intensity of positive-cell tumor was significantly lower than that of negative-cell tumor from pre-contrast to post-contrast images of the tumor. Internalization of SPIO-mPEG-cRGD into targeted cells were observed by in vitro and in vivo MR imaging studies.

Introduction

Molecular Imaging has recently been developed very rapidly and extensively in biotechnology [1]. Tumor-targeted drug delivery can enhance the effectiveness of chemotherapeutics while decreasing the systemic toxicity of these drugs. Iron oxide nanoparticles for MR imaging are often coated with poly(ethylene glycol) (PEG), which itself has been frequently used as a drug carrier because of its biocompatibility, water solubility nonimmunogenic, nonantigenic and protein resistant. There are some reports that macromolecules conjugated with cRGD peptides, were successfully recognized by $\alpha_v\beta_3$ integrins receptor and internalized into cells via $\alpha_v\beta_3$ integrin receptor-mediated endocytosis [2, 3]. In this study, we synthesized a new T_2-weighted contrast agent which has nanometer-size, hydrophilic, long-circulating PEG-coated and characterized SPIO particles that were tethered to cRGD (SPIO-mPEG-cRGD).

Methods

We test various cell lines with different levels of $\alpha_v\beta_3$ integrin over-expression: MCF-7, HT-29, A549 and HT1080. In addition, KB cell line was chosen as negative cell which low-expression $\alpha_v\beta_3$ integrin receptors. All cells were incubated with SPIO-mPEG-cRGD nanoparticles (0.3 mM Fe), washed by PBS buffer and scanned by 3.0 T MRI. Five nude mice (5 weeks old, female) were subcutaneous injected A549 and KB cells to the left and right lateral thigh of mice. MRI experiment was performed two weeks after tumor implantation, at which time the tumors were measured to be at least 0.5 cm in diameter. This method produces a high yield of tumor in the lateral thighs of nude mice.

Results and Discussion

SPIO-mPEG-cRGD nanoparticles were synthesized and characterized by DLS, FT-IR, 20 MHz relaxometer, NMR, SQUID, TGA, and TEM. Before cRGD peptide was conjugated with SPIO nanoaprticles, we pre-modified the surfaces of SPIO nanoparticles to improve the affinity of cRGD conjugating to the particles. Above all, the SPIO nanoparticles were synthesized by pyrolysis ferric-oleate complexes and coated oleic acid around the surface. And then the surface hydrophobic oleate of SPIO was replaced by hydrophilic mPEG derivative, mPPDA-silane. According to thiol-disufur bond exchange reaction, SPIO-mPPDA was conjugated with the bio-moiety of cRGD. The SPIO-mPEG-cRGD was performed by *in vitro* and *in vivo* MR imaging. The targeting of SPIO-mPEG-cRGD to $\alpha_v\beta_3$ integrin receptor positive cells (MCF-7, HT-29, A549 and HT1080) were confirmed by *in vitro* MR imaging, as shown in Figure 1. With the presence of the SPIO-mPEG-cRGD conjugates, the A549 and MCF-7 cell lines showed noticeable MR contrast. The internalization of SPIO-mPEG-cRGD into A549 cell tumor was also confirmed by *in vivo* study. Fig. 2 shows that T_2-weighted fast spin echo images of a tumor-bearing mice before (Fig. 2, left) and 0.5 h after (Fig. 2, right) intravenous administration of SPIO-mPEG-cRGD. The signal intensity of A549 cell tumor on the left was significantly darker than that of KB cell tumor. In other words, the nanoparticles were readily internalized into high $\alpha_v\beta_3$ integrin receptor-expressing tumor.

Conclusion

We have successfully prepared and characterized biocompatible superparamagnetic SPIO-mPEG-cRGD. They are hydrophilic, weakly cell-cytotoxic, and well-dispersed under physiological conditions. Moreover, SPIO-mPEG-cRGD have the ability to target and differentiate human cancer cells with $\alpha_v\beta_3$ integrin expression such as MCF-7, HT-29, A549 and HT-1080 and tumors as proven by *in vitro* and *in vivo* MR imaging studies. Therefore, SPIO-mPEG-cRGD can be potentially used as an MRI contrast agent for the detection of $\alpha_v\beta_3$ integrin expression cancers.

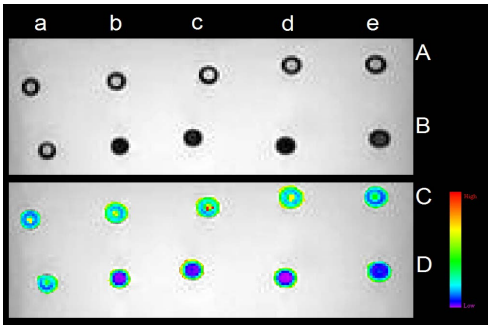

Fig 1. In vivo T_2-weighted MR images and color maps. (A) The blank cells. (B) After inculcation with SPIO-mPEG-cRGD, the enhanced images of different integrin-expressed cells. (C) The color maps of (A). (D) The color maps of (B). Cell line : (a) KB, (b) MCF-7, (c)HT-29, (d)A-549, and (e) HT-1080.

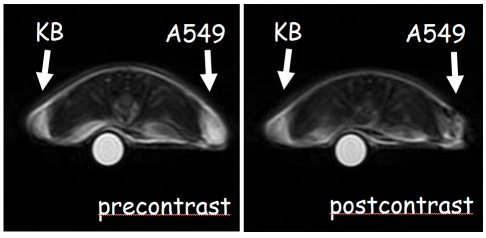

Fig 2. T_2-weighted images of pre- and post-injection (0.5 h) of SPIO-mPEG-cRGD.

Reference

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