Targeted MnFe2O4-Erbitux-CyTE777 nanoparticles toward high EGFR expressing cancer cells for in vitro and in vivo MR imaging

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Abstract

A multimodal target specific probe consisting of a biocompatible magnetic nanoparticle capable of altering the MR signal intensity, a NIR fluorochrome and a biological moiety that possess lock-and-key interactions can provide consistent *in vivo* imaging information with high sensitivity and spatial resolution. We have synthesized magnesium doped iron oxide nanoparticle as MR enhancer by thermal decomposition method. Post-synthesis surface modification with PEG expose the amine group which have been used for bioconjugation of cetuximab bearing near-infrared (NIR) fluorophore which can specifically target EGFR expressing tumor cell. The nanoparticle was characterized by TEM, SEM, FTIR, DLS, and zetapotential. *In vivo* optical imaging and *in vitro* and *in vivo* MR imaging studies demonstrate high specificity and sensitivity of these nanoparticles for EGFR. Furthermore, the biodistribution study also showed the high fluorescence signal in the EGFR positive tumor compared to the negative tumor.

Introduction

Technological limitations of imaging modality often hold back diagnosis and therapy of cancer. Having known through extensive clinical experience, it is widely accepted that early diagnosis may lead to cure or at least offer extended life to the cancer patient. Epidermal growth factor receptor (EGFR) are often over expressed in a wide variety of malignant cells including cancers of the colon, brain, breast, head and neck, pancreas, lung, and ovary. Consequently, EGFR is ideal target for receptor-based targeted therapy of cancer through the application of tumor-recognizing molecules (TRMs)[1]. Erbitux is chimeric monoclonal antibody specific for the EGFR. It has been safely used in human for the treatment of metastatic colorectal cancer (MCRC) and squamous cell carcinoma of head/neck cancers (SCCHN)[2]. Recent studies have demonstrated that Erbitux can be potentially employed for targeted drug delivery to tumor site. In addition, cetuximab has been the focus of a number of studies involving in vivo evaluation of EGFR. Generally, structural and functional information are required to get the complete insight of the cause and progress of cancer. However, a single imaging modality cannot provide all the information, given that, each imaging modality has its own specific strengths and weaknesses in respect to detection sensitivity, temporal resolution, spatial resolution, tissue penetration, signal-to-noise and quantitative accuracy. Herein, we report EGFR specific multimodal imaging agents, $MnFe_2O_4$-Erubitux-CyTE777 nanoparticles.

Materials and Methods

The monodisperse $MnFe_2O_4$-nanoparticles were obtained by thermal decomposition method. Post-synthesis ligand exchange reaction was carried out to replace hydrophobic oleic acid and oleylamine surfactants with mPEG-NH_2-silane in order to transform hydrophobic $MnFe_2O_4$ nanoparticles into hydrophilic ones. *In vitro* MR imaging was carried out using three EGFR positive cell lines (A-431, SKBR-3 and PC-3) with different levels of EGFR overexpression and Colo-205 cells as negative cell for control which has low level of EGFR expression. All cell lines were incubated with $MnFe_2O_4$-Erbitux (0.5 mM Fe), washed by PBS buffer and scanned by 3.0 T MRI. *In vivo* and whole body florescent imaging study was carried out in animal modal bearing subcutaneous tumor xenografts of A431 and COLO-205 in left and right lateral thigh of nude female mice. MR imaging studies were performed with a 3.0 T MR imager and a high-resolution animal coil. Whole body fluorescent images were carried out by IVIS spectrum imaging system.

Results and Discussion

To demonstrate the diagnostic potential of the $MnFe_2O_4$-Erubitux-CyTE777 as MR and optical contrast agent, we investigated in vitro and in vivo MR imaging. The T2-weighted in vitro MR images of cancer cell lines using $MnFe_2O_4$-Erubitux-CyTE777 was shown in Figure 1. The MR image of A431, SKBR-3, and PC-3 cells incubated with $MnFe_2O_4$-Erubitux-CyTE777 exhibited significantly high negative enhancement while negligible negative enhancement was observed in low EGFR expressing of COLO-205 cell line. We had further investigated negative contrast enhancement induced by $MnFe_2O_4$-Erubitux-CyTE777 in animal modal. Figure 2 showed control images of nude mice bearing subcutaneous tumor xenografts of EGFR-positive A431 and EGFR-negative COLO-205 cell line in right and left lateral thighs, respectively. After the tumor-bearing mice were pre-scanned for control images using 3.0-T MR imaging scanner, $MnFe_2O_4$-Erubitux-CyTE777 nanoparticles (15 mmol/kg) were I.V. injected via the tail vein into mice. Figure 2 illustrated negative contrast enhancement induced by $MnFe_2O_4$-Erubitux-CyTE777 at different point of time. Figure 3 showed typical whole body fluorescent images of nude mice bearing subcutaneous tumor xenografts of A431 and COLO-205 cell line at different time point after i.v. injection of 10 nmol $MnFe_2O_4$-Erubitux-CyTE777 nanoparticles. As showed in Figure 3, one hour after postinjection a strong fluorescence enhancement was observed in A431 tumor cells which remained elevated for over 120 min. Moreover, NIRF signal persisted for up to 24 hours, with a gradual attenuation in signal intensity in the tumor. In addition, we had performed ex vivo tissue- and organ-selective biodistribution study.

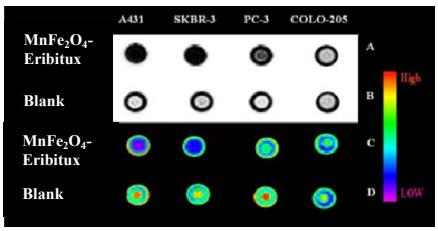

Figure 1. *In vitro* T_2-weighted MR images of different EGFR expressed cell line.

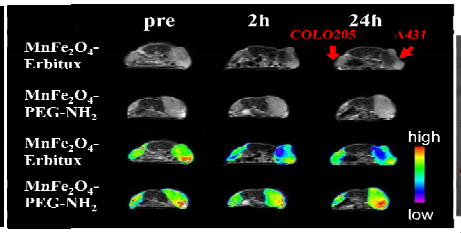

Figure 2. *In vivo* T_2-weighted MR images and the color mapped before and after injection of $MnFe_2O_4$-PEG-NH_2 nanoparticles (control) and $MnFe_2O_4$-Erbitux nanoparticles (15 mmol/kg).

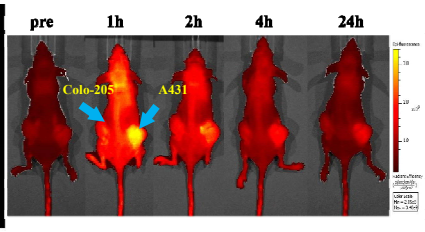

Figure 3. Mice bearing human epidermoid cancer cell (A431) and human colorectal cancer cells xenograft (COLO-205) were injected with $MnFe_2O_4$-Erbitux-CyTE777 nanoparticles.

Conclusion

In summary, we successfully synthesized $MnFe_2O_4$-Erubitux-CyTE777 nanoparticles for MR and optical imaging. The *In vitro* results revealed that this $MnFe_2O_4$-Erubitux –CyTE777 nanoparticles efficiently targets to EGF receptor expressing cells. In addition, $MnFe_2O_4$-Erubitux-CyTE777 nanoparticles had the ability to target A431 tumor cells *in vivo* MR imaging and *ex vivo* optical imaging studies. Thus, the $MnFe_2O_4$-Erubitux-CyTE777 nanoparticles can be potentially used as contrast agents for molecular MR imaging.

References

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