In vivo tracking of gastric stem cell by MRI using a newly synthesized iron-based contrast agent, $MnFe_2O_4$-PEG

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Abstract

We successfully synthesized and characterized a new iron-based MR contrast agent, superparamagnetic manganese ferrite nanoparticles conjugated with polyethylene glycol ($MnFe_2O_4$-PEG), for labeling gastric stem cell in vitro. The carcinogenetic potential of the stem cell, CS12, was well preserved following MR contrast labeling. In vivo MRI depicted not only the tumor growth but also intense T_2* effect over three weeks.

Introduction

Cell-based therapies may gain future importance in defeating different kinds of diseases, including cancer, immunological disorders, neurodegenerative diseases, cardiac infarction and stroke. Recent evidence suggests that stem cells may be the source of the mutant cells that give rise to cancerous tumors and maintain their growth [1,2]. We generated and characterized a novel human gastric cell line, CS12, derived from a putative human gastric stem cell/progenitor cell clone, KMU-GI2 [3]. The CS12 exhibited carcinogenic phenotypes and demonstrated that they are likely to carry chromosomal mutations affecting the expression of the HOXA gene. Therefore, HOXA expression may play a role in stem-cell gastric carcinogenesis by influencing progenitor self-renewal, proliferation, and differentiation [4]. In this study, we synthesized a new-MR T_2-weighted agent ($MnFe_2O_4$-PEG), and successfully labeled and tracked CS12 and KMU-GI2 with a 3.0 T clinical MR scanner (Sigma; GE) in vivo.

Methods

The monodisperse $MnFe_2O_4$ nanoparticles were obtained by thermal decomposition in hydrophobic solution at high boiling process. The $MnFe_2O_4$-nanoparticles surface was modified with mPEG-NH_2-silane ($MnFe_2O_4$-PEG). The $MnFe_2O_4$-PEG were synthesized and characterized by TEM, SQUID and FT-IR. The KMU-GI2 cell line, which lacks the carcinogenetic potential, was used as a control. The KMU-GI2 and CS12 were cultured with a KC medium. For cellular proliferation capacity, the cells were incubated with various concentrations of $MnFe_2O_4$-PEG for 3 and 24 hrs. Then they were reincubated for additional 3 days,added BrdU kit, and measured by ELISA. Cellular labeling was evaluated with MR imaging of labeled cell suspensions and Prussian blue staining histologically. Percentage change in signal intensity was normalized to the unlabeled cells by using the following equation: % change = $[(L - U)/U] \times 100\%$, where L = signal intensity of $MnFe_2O_4$-PEG labeled cells and U = signal intensity of unlabeled cells. In order to evaluate the carcinogenetic potential, six nude male mice were locally injected with labeled CS12 and KMU-GI2 into the right and left lateral thighs. In vivo MR imaging studies were performed 1, 2 and 3weeks after labeled cells injected using a 3.0 T clinical MR scanner and a high-resolution animal coil.

Results and Discussion

TEM analysis showed that the nanoparticles were well dispersed and coated with mPEG. The detection of saturation magnetization by SQUID magnetometry demonstrated that $MnFe_2O_4$-PEG had high saturation magnetization (78 emu/g) and small coercivity (about 4 G). Cellular proliferation were well preserved both in the labeled and unlabeled cells. This may indicate that toxicity of $MnFe_2O_4$-PEG is low and well tolerated. The intracytoplasmic particles stained with Prussian blue stain were observed with a labeling efficiency more than 95% (Figure 1). In vitro MR imaging of the CS12 cells incubated with various concentrations of $MnFe_2O_4$-PEG demonstrated that the signal intensity on the T_2-weighted images was reversely correlated with the concentration of contrast used for incubation (5%–91% decrease in signal intensity) (Figure 2). In vivo MR images of nude mice showed that tumor growth over time on the right side with $MnFe_2O_4$-PEG labeled CS12 cells implanted, but no tumor growth on the left with $MnFe_2O_4$-PEG labeled KMU-GI2 cells implanted(Figure 3). Moreover, the T_2* effect was still observed by MRI on both sides over this period of time (Figure 3b). We believe that this molecular imaging technique may contribute further understanding of carcinogenesis induced by gastric stem cell and it may be also beneficial to help gene or cellular therapy in the future.

Conclusion

We have successfully synthesized and characterized a novel iron-based MR contrast agent, $MnFe_2O_4$-PEG, for labeling gastric stem cell in vitro. Its carcinogenetic potential was well preserved following MR contrast labeling. In addition, tumor growth from the labeled CS12 cell and the T_2* effect can be efficiently detected over three weeks with in vivo MRI.

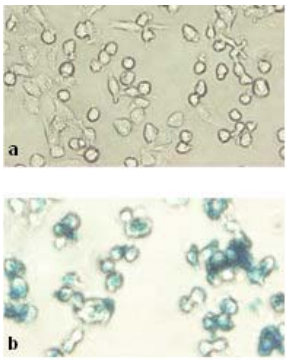

Figure 1. Microphotography of Prussian blue stained CS12 cells.
a. Unlabeled CS12, 100X.
b. Labeled CS12, 100X

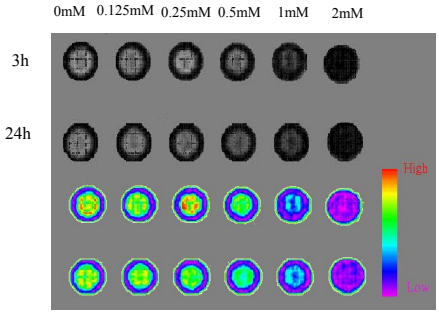

Figure 2. The in vitro MR imaging of various concentrations of $MnFe_2O_4$-PEG incubated with CS12 cells at 3 and 24 hours.

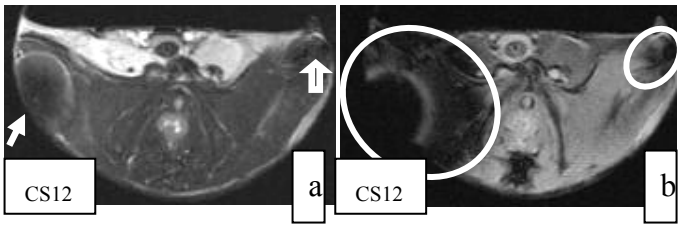

Figure 3. MRI of nude mouse 3 weeks post -injected with labeled CS12 and KMU-GI2 into the right and left lateral thighs, respectively. (a) a T_2-weighted image (TR /TE: 1500 /45msec; matrix 256 x192; Flap angle 90; FOV 30 mm; NEX 2; and slice thickness 2 mm) show tumor mass in right thigh (white arrow) and nil tumor with residual labeled cells in left thigh (white empty arrow). (b) a T_2*gradient-echo image (TR /TE: 300 /12msec; matrix 256 x192; Flap angle 12; FOV 30 mm; NEX 2; and slice thickness 2 mm) show prominent susceptibility effect around the injected regions (white circles)

References

[1] Varmus HE, Weinberg RA. Gene and Biology of Cancer: W H Freeman & Co.; 1992.
[2] Marx J. Cancer research. Mutant stem cells may seed cancer. Science 2003 Sep 5;301(5638):1308-1310.
[3] Yang YC, Wang SW, Hung HY, Chang CC, Wu IC, Huang YL, et al. Isolation and characterizationof human gastric cell lines with stem cell phenotypes. J Gastroenterol Hepatol 2007Sep;22(9):1460-1468.
[4] Yang YC, Wang SW, Hung HY, Chang CC, Wu IC, Huang YL, Lin TM, Tsai JL, Chen A, Kuo FC, Wang WM, Wu DC. CS12, a Human Gastric Epithelial Transformed Cell Line with High Tumorigenic Potential, Derived from Putative Gastric Stem/Progenitor Cells. European Journal of Gastroenterology & Hepatology 2008.