

Development of a novel target moiety of SPIO contrast agent for effectively targeting to pancreatic cancer cells *in vitro* and *in vivo*

YunMing Wang¹, ShouCheng Wu¹, and YuJen Chen¹

¹Department of Biological Science and Technology, National Chiao Tung University, HsinChu, Taiwan

Abstract

In this study, we developed MR-optical imaging contrast agents for the diagnosis and treatment of pancreatic cancer. A highly sensitive and specific T_2 weighted MR-optical imaging agent (SPIO-mAb-Cy777-mPEG NPs) has been synthesized. The SPIO NPs are used as MR enhancer, and Cy777 serves as optical imaging dye. Besides, the mAb has been used to target mucin4 protein which is overexpressed in pancreatic cancer. *Ex vitro* and *in vitro* experiments show significant negative contrast enhancement in mucin4 positive cell lines. As a result, we demonstrated that the T_2 weighted MR-optical imaging agent can specifically target mucin4 and can be potentially used for the detection of pancreatic cancer.

Introduction

Pancreatic cancer is a major cause of death among all cancers in both the USA and Europe. It is very difficult to diagnose and is the most deadly of all major cancer diseases with a 5 year survival rate of only 5% [1]. Recent studies have shown that mucin4, an O-glycoprotein of the membrane-bound mucin gene family, is abundantly expressed in pancreatic adenocarcinoma [2]. Therefore, mucin4 is a potential biomarker for pancreatic cancer detection. Herein, we report MR-optical contrast agents for early and specific detection of pancreatic cancer.

Materials and Methods

All cell lines (HPAC, BxPC3, and Panc-1) were incubated with contrast agents (10 $\mu\text{g}/\text{mL}$) for 4 hrs at 37 °C. The cells were observed using a laser scanning confocal imaging system. The contrast agents (10 mg/kg) were infused to the nude mice bearing HPAC and Panc-1 tumor cells. MR imaging was performed on a 7.0 T MRI system (TR/TE = 3,000/90). Images were acquired at pre-injection and various time points post-injection. The contrast enhancement (%) was calculated by the following equation (enhancement (%) = (SI_{post} - SI_{pre}) / SI_{pre} × 100.) Optical imaging was acquired at pre-injection and various time points post-injection using an IVIS system. (the wavelength of excitation and emission was 745 nm and 820 nm)

Results and Discussion

To characterize the *in vitro* specificity of contrast agents for the pancreatic tumor cells, the uptake experiment was studied using a laser scanning confocal imaging system. The mucin4-expression cancer cells, HPAC and BxPC3 cells, were chosen as target cancer cells, whereas Panc-1 cancer cells were used as a control cells. As shown in Figure 1 (A), the maximum fluorescence intensity can be observed in HPAC cells and the minimum in Panc-1 cells, reflecting the mucin4 expressing levels. *In vivo* MR images (Figure 1 (B)) show that negative contrast enhancement was significantly observed in HPAC tumor (-70.5%), but only slight observed in Panc-1 tumor (-19.6%), indicating the little accumulation of the contrast agents. To further investigated the optical imaging ability (Figure 1 (C)). A strong fluorescence enhancement is observed in HPAC tumor. However, it is not observed in the Panc-1 tumor. These data was consistent with MR imaging studies and indicated that the strong signals resulted from the specific binding of contrast agents to the mucin4 expressing tumors.

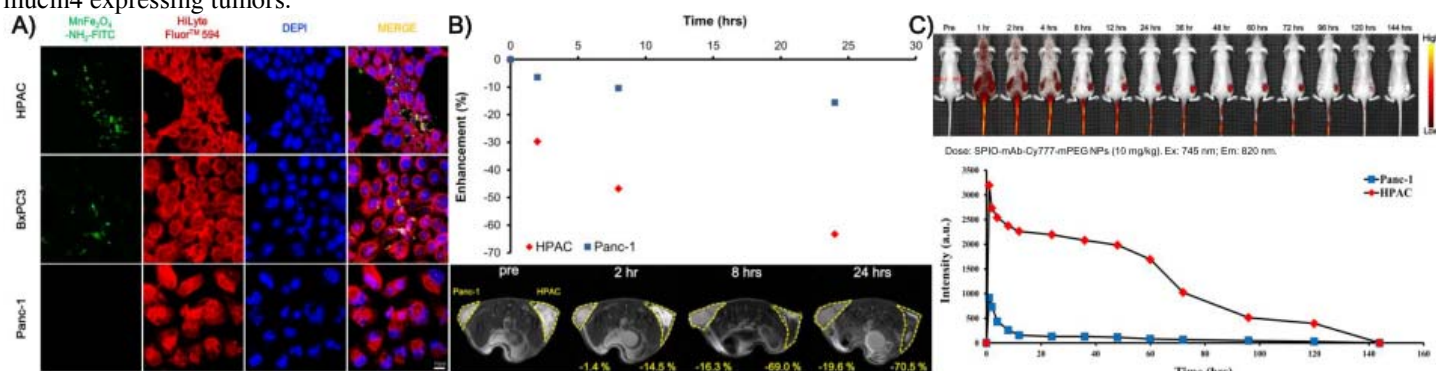


Figure 1. A) Confocal microscopy images of negative control cell lines incubated with SPIO-mAb-FITC-mPEG NPs (10 $\mu\text{g}/\text{mL}$) for 4 hrs at 37 °C. Cytoplasm (stained with HiLyse Fluor™ 594 in red), nuclei (stained with DEPI in blue). B) T_2 -weighted MR images of nude mice bearing subcutaneous tumor xenografts of Panc-1 (left) and HPAC (right) tumor cells before and after injection of SPIO-mAb-Cy777-mPEG (10 mg/kg). Images were acquired at pre-injection and 2.0, 8.0, and 24.0 hrs of post-injection. C) *In vivo* optical images of nude mice bearing subcutaneous tumor xenografts of Panc-1 (left) and HPAC (right) tumor cells after intravenous injection of SPIO-mAb-Cy777-mPEG NPs (10 mg/kg). Images were acquired at pre-injection and various time points (1.0, 2.0, 4.0, 8.0, to 144 hrs) post-injection.

Conclusion

In summary, we successfully synthesized contrast agents for MR-optical imaging. The *in vitro* results confirming that the contrast agents can specifically targeted to mucin4 expressing cells. Thus, the SPIO-mAb-Cy777-mPEG NPs could be potentially used as contrast agents for MR imaging and reducing the off-targeted. Therefore, we further anticipated that this approach could be exploited for pancreatic cancer therapy.

References

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