Polymer-bound NIRF Probe to Improve Colorectal Cancer Detection

Ayelet David, Inga Kogan-Zviagin, Moran Golan

Department of Clinical Biochemistry and Pharmacology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva, 84105, Israel. ayeletda@bgu.ac.il

ABSTRACT SUMMARY
We report herein the development of new polymeric imaging probe that allows non-invasive identification of colorectal cancer (CRC) tumors from the luminal aspect of the colon. The HPMA-based NIRF polymeric probe can detect the presence of human colorectal tumors that are either exposed or non-exposed to the colon lumen, when applied intracolonically to LS174T- and HT29-tumor bearing mice models. This unique probe can be applied in conjunction with colonoscopic procedure and serve as a helpful tool in guiding selective removal of polyps discovered during colonoscopy.

INTRODUCTION
Developing optical imaging tools that can be administered non-parenterally may considerably improve the safety and accuracy of CRC cancer diagnostics. We report herein the design of HPMA-based polymeric carrier labeled with near-infrared fluorescence (NIRF) dye for targeting CRC tumors that over-express the underglycosylated mucin-1 (uMUC-1) antigen. uMUC-1 is one of the early hallmarks of tumorigenesis. While in normal tissues mucin-1 is heavily glycosylated, in neoplastic tissues MUC-1 is in an underglycosylated form, exposing the peptide core of the tumor-associated uMUC-1 and reveals epitopes, which in the normal cell are masked (1). This feature makes it an attractive biomarker for targeting polymer-based NIRF probes to cancerous tissues. The synthetic EPPT1 peptide has been previously shown to specifically target in vivo breast carcinomas that overexpress uMUC-1 following intravenous administration (2), and thus may serve as targeting ligand to increase the efficiency of the diagnostic probe.

EXPERIMENTAL METHODS
Two HPMA precursor copolymers having active ester (p-nitrophenyl) groups and bearing either fluorescein-isothiocyanate (FITC) or NIRF dye (IR783) were synthesized by random radical precipitation copolymerization, and then conjugated with N-term lysine harboring EPPT1 (YCAREPPTRTFAYWG) peptide for targeting uMUC-1 expressed in neoplastic tissues. The targeted FITC-labeled copolymer, P-(EPPT1)-FITC, was investigated in vitro for its ability to bind human CRC cells and human CRC tissue specimens. The NIRF-labeled copolymer, P-(EPPT1)-IR783 was further assessed in vivo for its ability to detect colonic lesions in LS174T- and HT29-tumor bearing mice by IVIS Lumina imaging system, following intra-luminal administration of the copolymers with the guidance of a mini endoscope.

RESULTS AND DISCUSSION
P-(EPPT1)-FITC demonstrated superior binding to CRC cells that over-express the uMUC-1 antigen and exhibited selectivity towards human CRC tissue specimens when compared to adjacent normal tissues of the same patient. When applied intracolonically, P-(EPPT1)-IR783 accumulates significantly in cancerous tissue relative to the adjacent normal mucosa of HT29 and LS174T tumor bearing mice, and demonstrates higher signal intensities in colonic tumors when compared to the non-targeted P-(GG-OH)-IR783 probe (without EPPT1) (Fig. 1). Interestingly, we found that the P-(GG-OH)-IR783, can accumulate specifically at tumor sites as well. The cancer-specific uptake and retention of P-(GG-OH)-IR783 was not mediated by the organic anion transporting peptides (OATPs). The significant accumulation of the uMUC-1-targeted probe was partially inhibited by OATPs inhibitor.
Fig.1: Representative fluorescence images of healthy and cancerous tissues of LS174T tumor bearing mice, treated with P-(EPPT1)-IR783 (25 µg), P-(GG-OH)-IR783 (25 µg) and with an equivalent dose of free IR783-S-Ph-COOH (2.5 µg), when applied intra-colonically with the guidance of a mini endoscope.

CONCLUSION
The polymer-bound NIRF probe can successfully detect solid tumors at the GI tract following intracolonic administration, and may be applied in conjunction with colonoscopic procedure to improve the sensitivity of colonoscopy for polyp detection.

REFERENCES

ACKNOWLEDGMENTS
This study was supported by the Bio Medical Photonics (BMP) Consortium within the Magnet program of the Israeli Ministry of Industry and Commerce.