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In vivo targeting of colonic dysplasia on fluorescence endoscopy with near-infrared octapeptide

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Abstract

Objective—To demonstrate a near-infrared peptide that is highly specific for colonic adenomas on fluorescence endoscopy in vivo.

Design—A 3 mm diameter endoscope was adapted to deliver 671 nm illumination and collect NIR fluorescence (696–736 nm). Target (QPIHPNNM) and control (YTTNKH) peptides were labeled with Cy5.5, a near-infrared dye, and characterized by mass spectra. The peptides were topically administered separately (100 μ M) through the endoscope's instrument channel into the distal colon of *CPC;Apc* mice, genetically-engineered to spontaneously develop adenomas. After 5 minutes for incubation, the unbound peptides were rinsed off, and images were collected at a rate of 10 per second. Regions-of-interest were identified around the adenoma and adjacent normal-appearing mucosa on white light. Intensity measurements were made from these same regions on fluorescence, and the target-to-background ratio (TBR) was calculated.

Results—We achieved an image resolution of 9.8 μ m and field-of-view of 3.6 mm at a distance of 2.5 mm between the distal end of the instrument and the tissue surface. On mass spectra, the experimental mass-to-charge ratio for the Cy5.5-labeled target and control peptides agreed with expected values. The near-infrared fluorescence images of adenomas revealed individual dysplastic crypts with distorted morphology. By comparison, only amorphous surface features could be visualized from reflected near-infrared light. The average TBR for adenomas was found to be 3.42 ± 1.30 and 1.88 ± 0.38 for the target and control peptides, respectively, $p=0.007$.

Conclusion—We demonstrate a near-infrared peptide that is highly specific for colonic adenomas on fluorescence endoscopy in vivo and achieved sub-cellular resolution images.

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Competing Interests: The University of Michigan has filed a provisional patent on behalf of authors SJM, BPJ, and TDW on the peptide presented in this study.

Author Contributions:

- Designed research – ZL, SJM, BPJ, TDW
- Performed research – ZL, SJM, BPJ
- Contributed new reagents or analytic tools – SJM, BPJ
- Analyzed data – ZL, SJM, BPJ, TDW
- Wrote the paper – ZL, SJM, BPJ, TDW

Keywords

colorectal adenomas; colonoscopy; imaging; molecular pathology; peptides

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Introduction

Colorectal cancer is one of the most common malignancies in industrialized countries.¹ This disease has a long period of dormancy in a pre-malignant state (dysplasia) that provides a window of opportunity for early detection.² Colonoscopy is a reliable method for detecting dysplasia, and is widely used for screening. While this method is sensitive to gross architectural changes, such as mass effect, texture variations, and color changes, this approach is not sensitive to the expression of molecules by cells and tissues that can reveal important details about mucosal function, including cell surface receptors, extracellular matrix proteins, and metabolite levels. A number of clinical studies have shown a significant miss rate of ~20% for the detection of adenomas.^{3,4,12} Furthermore, flat and depressed lesions, which are more difficult to detect on white light colonoscopy, may represent over 25% of all dysplastic lesions in some patient populations, and may confer a higher risk for malignancy.^{5–8} High resolution (white light) endoscopy (HRE) facilitates detection of neoplastic lesions from reflections of the mucosal surface.⁹ Advanced imaging techniques, such as near-infrared (NIR) fluorescence endoscopy with targeted contrast agents, can further enhance detection of these lesions. Improvement may result from significantly increasing contrast between diseased and normal tissue, validating in situ cancer targets, monitoring clonal evolution of progenitors, characterizing risk for cancer progression, and assessing likelihood for response to therapy.

Targeted imaging with NIR fluorescence provides a red-flag detection strategy that addresses limitations of other advanced techniques based on use of visible light, such as autofluorescence imaging (AFI) and narrow-band imaging (NBI).¹⁰ AFI delivers blue light (395–475 nm) incident on colonic mucosa to produce fluorescence in the visible regime (490–625 nm).¹¹ Prospective randomized clinical trials have been performed using tandem colonoscopies to compare the yield for detecting adenomas on AFI versus HRE. The results show no significant reduction in the adenoma miss rate.¹² This finding may be attributed to a lack of specificity of the endogenous fluorophores measured for dysplasia. NBI uses special optical filters to reduce the spectral bandwidth of the blue (400–430 nm), green (520–560 nm), and red (580–620 nm) components of white light for illuminating the mucosa.¹³ This adaptation increases the contrast between the epithelial surface and adjacent vasculature by enhancing the effect of hemoglobin absorption. In randomized controlled clinical trials, NBI did not result in a higher yield for the detection for adenomas.¹⁴ Thus, contrast enhancement based on hemoglobin alone is not likely to improve the adenoma miss rate.

Peptides are promising for in vivo use as targeting agents to visualize gene expression.¹⁵ In addition to high clonal diversity, small size, and broad compatibility with fluorescence dyes, peptides have rapid binding kinetics that are compatible with real time in vivo “immunohistochemistry.” We have previously demonstrated the use of the FITC-labeled peptide “VRPMLQ” for specific binding to colonic dysplasia on confocal endomicroscopy.¹⁶ However, imaging performed with visible light results in reduced contrast that is limited by hemoglobin absorption, autofluorescence background, and tissue scattering. By comparison, NIR fluorescence (spectral range 665–900 nm) is much less sensitive to these factors, and development of this approach can significantly improve imaging performance.¹⁷ Moreover, confocal imaging as well as other methods of optical sectioning, such as multi-photon, samples only a tiny FOV on the order of ~100 microns,¹⁸ a

diameter that is inadequate for rapid surveillance of large surfaces in hollow organs which has dimensions on the millimeter to centimeter scale. The collection of endoscopic images in the NIR regime with sub-cellular resolution can provide sharp margins to assist the physician by accurately guiding endoscopic mucosal resection.¹⁹

NIR fluorescent probes that identify colorectal cancer have been demonstrated previously in mouse xenograft models. A activatable cathepsin B probe was shown to visualize mouse CT26 colorectal adenocarcinoma cells implanted orthotopically in the colon of mice, and imaged in vivo with a fiber optic catheter that collected wide-field images in the 690–800 nm regime with a resolution of 7 lp/mm.²⁰ In addition, human HT29 colorectal cancer cells were visualized 2 hours after intravenous administration of a cyclic peptide labeled with Cy5.5.²¹ We have isolated the peptide QPIHPNNM using phage display technology in a genetically-engineered *CPC;Apc* mouse model that spontaneously develops colonic dysplasia based on the somatic inactivation of the *Apc* gene.²² This model is highly representative of pre-malignant human disease, as *Apc* mutations are seen in most (85–90%) colorectal cancers.²³ Here, we describe the design, construction, and validation of a near-infrared (NIR) labeled peptide and the development of a wide-field imaging instrument that visualizes this probe with sub-cellular resolution in real time. We then demonstrate the use of this novel, integrated imaging strategy to visualize individual pre-malignant (dysplastic) crypts in vivo, an unprecedented level of performance that can be translated into the clinic to improve methods for early detection of colorectal cancer.

Materials and Methods

NIR-labeled fluorescent peptides

We have previously screened a highly specific peptide that binds to colonic dysplasia in the *CPC;Apc* mouse using phage display technology.²² This octapeptide has amino acid sequence QPIHPNNM, and was conjugated with Cy5.5 (Lumiprobe, LLC, Hallandale Beach, FL) on the N-terminus via an amino-hexanoic acid linker using NHS activation chemistry. The peptide was prepared using solid-phase synthesis by applying standard fluorenylmethoxycarbonyl (Fmoc) chemistry on Rink amide MBHA amide resin (Anaspec, CA).²⁴ Cy5.5-NHS ester was conjugated to the N-terminus of the peptide in mixed DCM/DMF (1:3) at pH 9 adjusted with diisopropylethylamine (DIEA) overnight.²⁵ Side-chain protecting groups were removed with a mixture containing trifluoroacetic acid (TFA)/triisopropylsilane (TIS)/ water (95:2.5:2.5, V/V/V). The crude product was purified using semi-preparative HPLC (C-18, Waters) with water-acetonitrile gradient mobile phase containing 0.1% TFA. The products were analyzed with HPLC System (Waters Breeze, 2489) using a C-18 analytical column and Q-TOF mass spectrometer (Agilent Technologies, Inc.; Santa Clara CA). To investigate the specificity of the target peptide, an unrelated peptide YTTNKH was selected randomly for use as a control. This peptide was synthesized, labeled with Cy5.5, purified and analyzed, as described above.

NIR fluorescence imaging system

The schematic of the NIR endoscopic imaging system is shown in Fig. 1A. A diode-pumped solid state laser (TechnicaLaser Inc, Winter Park, FL) with $\lambda_{ex} = 671$ nm is used to excite the Cy5.5 labeled peptides. The intensity is tunable over a range from 1 to 100 mW. The incident beam is expanded to 3 mm in diameter by a pair of relay lenses with focal lengths of $f_1 = 30$ and $f_2 = 50$ mm (Thorlabs Ltd, Newton, NJ) to fill the aperture of a fluid light cable that is coupled to a Hopkins® II rigid endoscope (Karl Storz, Goleta, CA).⁹ This pediatric urethroscope (Karl Storz Veterinary Endoscopy, Goleta, CA) has a ~3 mm outer diameter and a 3 Fr instrument channel. Fluorescence images collected by the endoscope are transmitted by another set of relay lenses onto a CCD detector that has dimensions of

8.7×6.9 mm² and 1388×1040 pixels (Carl Zeiss MicroImaging, LLC, Thornwood, NY). We use a zoom lens system (NT58-440, Edmund Optics Inc., Barrington, NJ) with a tunable focal length of 20–100 mm, F#1.6–16 aperture to magnify the image to fill the dimensions of the CCD. The resolution of this system was measured using a standard imaging target (USAF-1951, Newport Corporation, Irvine, CA). Each frame is collected in 100 ms (10 frames per second), and transferred to the computer by a fire wire connection. The optics and detector are contained within a custom housing made of light weight polymer. A filter wheel is incorporated into the handle of the housing to quickly switch between collection of reflectance and fluorescence images. A band pass filter was used to allow only NIR fluorescence from $\lambda_{em} = 696$ to 736 nm (FF01-716/40-25, Semrock, Inc., Rochester, NY) to be detected.

***CPC;Apc* mouse model of colonic dysplasia**

Mice were cared for under the approval of the University Committee on the Use and Care of Animals (UCUCA) at the University of Michigan. The *CPC;Apc* mice are genetically engineered with a Cre recombinase under the control of the Cdx2 promoter (CDX2P-9.5NLS-Cre) and a floxed allele of the APC gene.²⁶ This Cre-regulated somatic mutation in one of the Apc alleles causes dysplasia to develop spontaneously in the distal colon of the mouse.²⁷ Adenomas in this region can be easily visualized on endoscopy. All mice were housed in specific pathogen-free conditions and supplied water ad libitum throughout the study. Animals ranged in age from 6 to 9 months.

In vivo NIR fluorescence imaging

Anesthesia was induced and maintained with inhaled isoflurane mixed with oxygen via a nose cone at a dose of 4% and 2% at a flow rate 0.5 liter/min, respectively. Prior to peptide administration, the colon of each mouse was cleaned with a tap water lavage to remove debris. The conventional white light endoscope was used first with a color camera to identify the presence of adenomas. If adenomas were absent, the peptide was not administered. Otherwise, the colon was then rinsed with water until all of the visible mucous was removed. The Cy5.5-labeled peptides were then delivered at a concentration of 100 μ M in 1X PBS containing 2.5% DMSO (diluted in phosphate buffered saline) through the instrument channel. The peptides were allowed to incubate for 5 minutes, and then the colon was cleansed 3 times with a tap water to remove any unbound peptide. Prior to imaging, the colon was inspected for residual peptide solution, and when clean, the colon was insufflated with air for imaging. Fluorescence images were collected using Axiovision 4.8.1 software. A total of n = 5 mice each were used for the target and control peptides. The length of the colon inspected was ~5 cm.

Fluorescence Image Analysis

Images collected during endoscopy were recorded as .zvi files and converted into .tiff images using Axiovision Lite software. Consecutive white light and fluorescence images for each adenoma were analyzed. Regions of interest (ROI) around the adenoma and adjacent normal-appearing mucosa were defined on the color and fluorescence images using Labview®.²² The white light image was utilized to draw an ROI around the adenoma or adjacent normal appearing colonic mucosa which was then superimposed onto the fluorescent image. Mean gray scale values were calculated for each ROI, and an adenoma/normal colon, or a target-to-background (TBR) ratio, value was then calculated for each adenoma. The mean fluorescence intensities were calculated from each ROI to determine the ratio of the average intensities for the adenomas to that from adjacent normal-appearing mucosa, or TBR.

Peptide immunohistochemistry

All specimens were obtained with approval from the University of Michigan Institutional Review Board (IRB). Freshly resected specimens of human colonic mucosa were obtained either from surgical procurement or pinch biopsy during colonoscopy and immediately frozen in OCT and sectioned with 10 μm thickness using Microm-HM-550 Microtome (EquipNet, Inc. Canton, MA). The sections were then air dried, washed with PBS 1X, and incubated with the Cy5.5-labeled peptides at a concentration of 10 μM for 15 min at room temperature. The sections were washed 3X for 1 min in PBS and fixed with 4% paraformaldehyde for 10 min. After fixation, the sections were washed again (1X) and mounted with ProLong Gold reagent with DAPI (Invitrogen, Carlsbad, CA). The sections were imaged under a confocal microscope (Leica TCS SP5 Microsystems, Bannockburn, IL). Adjacent sections were sent for routine histology. The average intensity from the entire field-of-view of each image was calculated using Matlab (Mathworks, Natick, MA).

Statistics

Two-tailed independent samples t-tests (significance level at $\alpha = 0.05$) were used to determine statistical significance between the TBR for the QPI-Cy5.5 and YTT-Cy5.5 peptides binding to colonic adenomas in the *CPC;Apc* mice. All statistical computations were processed either using statistical software (SPSS 15.0.; SPSS, Chicago, IL) or Minitab (Minitab Inc. State College, PA).²⁸ For the ex-vivo peptide immunohistochemistry study, the nonparametric Kruskal-Wallis test was used to determine if observed differences are statistically significant among all three pathological classifications. The nonparametric Mann-Whitney test was used for comparisons between two independent samples, and differences with $p < 0.05$ were considered significant.

Results

The schematic of the NIR fluorescence imaging system is shown in Fig. 1A. The distal end of the endoscope is ~ 3 mm in diameter, and delivers excitation at $\lambda_{\text{ex}} = 671$ nm, shown in Fig. 1B. A custom polymer housing contains a filter wheel to provide both reflectance and fluorescence images, and has a handle for holding the instrument, shown in Fig. 1C. Using a standard target, a resolution of 9.8 μm (group 5, element 5, 50.8 lp/mm), highlighted by the red oval in the inset in Fig. 1D, was measured at a distance of 2.5 mm between the distal end of the instrument and the tissue surface. This is a typical dimension used for in vivo imaging, and results in a FOV with diameter of 3.6 mm.

The chemical structure of the NIR-labeled target peptide QPIHPNNM, hereafter QPI-Cy5.5, is shown in Fig. 2A. The peptide sequence (black) is separated by a linker (blue) from the Cy5.5 (red) label to prevent steric hindrance. On mass spectra, shown in Fig. 2B, the experimental m/z (mass units) measured for the QPI-Cy5.5 peptide is 1668.91, which agrees with the expected molecular mass of 1668.91. The chemical structure of the NIR-labeled control peptide YTTNKH, hereafter YTT-Cy5.5, is shown in Fig. 2C. On mass spectra, shown in Fig. 2D, the experimental m/z (mass units) measured for the YTT-Cy5.5 peptide is 1756.8, which agrees with the expected result of 1755.95. The purity of the final synthesized products was $>95\%$ for each NIR-labeled peptide. The fluorescence emission spectra with 671 nm excitation, shown in Fig. 2E, for QPI-Cy5.5 (red) and YTT-Cy5.5 (green) at a concentration of 10 μM in 1X PBS reveal peaks at $\lambda_{\text{em}} = 709$ nm and 708 nm, respectively. The spectral range for both NIR-labeled peptides is approximately 680 to 900 nm.

NIR fluorescence images were collected endoscopically in real time (10 frames per second) from the colon of *CPC;Apc* mice in vivo. The white light image, shown in Fig. 3A, reveals spontaneous colonic dysplasia developing in the left half of the image. The NIR reflectance

image, shown in Fig. 3B, depicts amorphous surface texture from dysplasia, and reveals several bright regions of specular reflection. The corresponding fluorescence image, shown in Fig. 3C, reveals significant contrast enhancement from binding of the QPI-Cy5.5 peptide to dysplasia but not to adjacent normal-appearing colonic mucosa. Note that the margins between dysplasia and normal mucosa are quite sharp in comparison to that on the white light and reflectance images. The fluorescence images from the same region of mucosa with administration of the control peptide YTT-Cy5.5, shown in Fig. 3D, and with no peptide (autofluorescence), shown in Fig. 3E, reveal negligible signal. On an expanded view of the yellow box in Fig. 3C, heterogeneous, oval structures (arrows) with dark central regions representing individual crypts can be appreciated, shown in Fig. 4A, magnification 4X. The corresponding histology (H&E), shown in Fig. 4B, reveals crypts with similar morphology (arrows). Also, typical features of dysplasia, including elongated nuclei, disorganized crypt structure, hyperchromaticity, and crowded lamina propria, similar to that found in human disease can be seen, scale bar 100 μ m.

There were a total of $n = 8$ adenomas evaluated with the target peptide and $n = 9$ with the control peptide. All adenomas were visible on both white light reflectance and NIR fluorescence. The size of the lesions were not measured on endoscopy. The average TBR measured from the respective regions-of-interest (ROI) for dysplasia and for the surrounding normal-appearing colonic mucosa for QPI-Cy5.5 was 3.42 ± 1.30 and that for YTT-Cy5.5 was 1.88 ± 0.38 . The distribution of TBR for individual dysplastic lesions with QPI-Cy5.5 ($n = 8$) and YTT-Cy5.5 ($n = 9$) is shown in Fig. 5. The mean of each group is represented by the horizontal line, and one standard deviation from the mean is depicted by the vertical line. Non-parametric Mann-Whitney independent samples analysis showed that the fluorescence intensity associated with binding of the target peptide to dysplasia was greater than that of the control with significance, $p = 0.007$.

We also evaluated the specificity of the QPI-Cy5.5 peptide binding to human colonic neoplasia to assess the potential for future clinical application. In Fig. 6A, the QPI-Cy5.5 (target) peptide demonstrates no binding to normal colonic mucosa ($n = 7$), but consistently stains dysplasia ($n = 8$), shown in Fig. 6B, and adenocarcinoma ($n = 10$), shown in Fig. 6C, scale bar 50 μ m. The YTT-Cy5.5 (control) peptide did not stain any of the specimens, including normal, dysplasia, and adenocarcinoma, shown in Figs. 6D–F, respectively, scale bar 50 μ m. The corresponding histology (H&E) for normal, dysplasia, and adenocarcinoma is shown in Fig. 6G–I for comparison, scale bar 200 μ m). In total, the QPI-Cy5.5 peptide was found to selectively stain dysplasia in 6/8 and adenocarcinoma in 8/10 sections of human specimens. The QPI-Cy5.5 peptide did not stain any of the 7 sections of normal colonic mucosa. YTT-Cy5.5 did not stain either normal or dysplasia, and was found to bind minimally to 5/10 adenocarcinoma sections. The mean NIR fluorescence intensities on immunohistochemistry for QPI-Cy5.5 peptide binding to normal, dysplasia and adenocarcinoma were 1.5 ± 1.0 , 10.7 ± 3.3 and 9.8 ± 2.4 AU, respectively. The resulting intensity ratio for dysplasia and adenocarcinoma was 7.1 ($p < 0.0049$) and 6.5 ($p < 0.0168$) relative to that for normal colonic mucosa. A comparison of the mean fluorescence intensities between dysplasia and adenocarcinoma using QPI-Cy5.5 peptide was not significantly different, $p = 0.41$. The TBR for binding by the QPI-Cy5.5 peptide in comparison to the YTT-Cy5.5 peptide was 26.7, 2.9, and 1.8 for dysplasia, adenocarcinoma and normal mucosa, respectively.

Discussion

Here, we demonstrate a novel method for targeted detection of colonic dysplasia in real time using a highly-specific peptide in vivo and a wide-field NIR fluorescence endoscope. We achieved a sub-cellular system resolution of $< 10 \mu$ m, representing a significant improvement

over previous NIR instruments,^{29,30} and were able to visualize individual dysplastic crypts and sessile lesions with well-defined features and sharp margins. The ability to attain this level of performance in hollow organs of the digestive tract has potential implications for future screening and early detection of cancer, including proximal lesions. The fast binding kinetics of the peptide allows for imaging to be performed within only a few minutes after administration, a time interval that is compatible with the busy work flow in the clinic. Topical application provides local disease contrast enhancement without increased background from the probe accumulation, which occurs in circulation with intravenous administration.^{31,32} Other molecular probe platforms, such as antibodies³³ and activatable probes,³⁴ have much slower onset and longer clearance, requiring hours (or even days) to reach peak effect, increasing background and limiting utility for diagnostic imaging.

Identifying pre-cancerous mucosa in the colon using targeting molecules, such as peptides, is a promising direction for improving methods of early detection of colorectal cancer, and addresses limitations of conventional colonoscopy for identifying lesions that do not exhibit gross morphological changes. Integration of molecular probes that home to diseased tissue with high resolution imaging instruments could greatly improve diagnostic accuracy for missed lesions of both polypoid and non-polypoid morphology, including right sided sessile serrated adenomas.^{35,36} In addition, wide-field fluorescence guidance may greatly impact the clinician's ability to perform endoscopic resection of neoplastic lesions by accurately defining the margins between diseased and healthy tissue. The use of an exogenous agent during surveillance colonoscopy will most likely be performed first in a high-risk patient population, such as those who have a first-degree relative with colorectal cancer, FAP, and HNPCC (Lynch Syndrome).³⁷ This peptide may also improve our ability to visualize flat and depressed lesions that occur in the setting of ulcerative colitis. We also evaluated binding of the QPI-Cy5.5 peptide on excised human specimens to support regulatory efforts for a future Phase 1 clinical study. The target peptide achieved a slightly higher TBR for dysplasia than for adenocarcinoma, a result that is consistent with this peptide being selected against dysplasia via phage display.²²

The use of NIR fluorescence has significant advantages over that of visible for performing in vivo imaging. We observed much less background in these fluorescence images in comparison to that found in our previous study with a FITC label.²² In addition, the resolution is far better than that of NIR endoscopes that use a fiber-optic bundle, which are limited by pixilation artifact.³⁸ NIR systems have also been developed for intra-operative imaging, but these instruments are large and not minimally invasive.^{39,40} Furthermore, NIR tomography has been used to image hollow organs, but this approach is limited by light penetration depth and multiple air-tissue interfaces.⁴¹ In this study, the use of a rigid endoscope limits visualization of the right side of the colon. Recent outcomes studies have found that conventional colonoscopy does not reduce the incidence or mortality of proximal colorectal cancers.^{42–45} We are currently working with industrial partners to develop NIR detection capabilities on standard medical endoscopes for future clinical application of this technology for right-sided lesions.⁴⁶ In addition, image quantification can be improved by collecting the fluorescence and reflectance images simultaneously and then ratioing to correct for motion artifact and variation in intensity with proximity and angle of the endoscope relative to surface geometry.³⁸

Our study showed that all of the adenomas in the genetically-engineered mouse model were detected in vivo. Moreover, peptide binding was observed ex vivo on 6 out of 8 human specimens, motivating a future clinical trial where the true sensitivity can be measured. In the future, this technology can be translated to the clinic to perform high resolution molecular imaging in hollow organs. In addition to the colon, this integrated strategy can be applied to the biliary tract, bladder, cervix, esophagus, lung, stomach, and oropharynx. The

system can be adapted for imaging of multiple spectral bands by adding the appropriate lasers, optics, and filters.⁴⁷ Some of the unique advantages of this integrated platform can also realized for longitudinal pre-clinical studies by imaging the deep red region of the visible spectrum and by using other targeting molecules and optical reporters (fluorescent proteins expressed by genetically-engineered mice). Moreover, systemic administration of the NIR-labeled peptides can generate fluorescence that can potentially be detected up to several centimeters below the mucosal surface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FOV	field-of-view
HRE	high resolution endoscopy
NIR	near-infrared
TBR	target-to-background ratio

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Summary Box

What is already known about this subject

- Conventional white light colonoscopy is sensitive to gross architectural changes and non-specific features of dysplasia, such as mass effect, texture variations, and color changes.
- The miss rate for detection of grossly visible and flat & depressed lesions on standard colonoscopy can be as high as 25% for all dysplastic lesions.
- Autofluorescence (AFI) and narrow-band (NBI) imaging have not been found to improve the yield of adenoma detection in prospective, randomized clinical trials.

What are the new findings

- Near-infrared labeled peptides can bind to colonic adenomas in vivo with high specificity and rapid binding kinetics, features compatible with practical clinical use.
- Novel near-infrared instrument can collect fluorescence images over endoscopic field-of-view in real time with sub-cellular resolution thus achieving sharp margins to guide endoscopic mucosal resection.
- Individual dysplastic crypts can be visualized with peptides labeled with near-infrared contrast agents.

How might it impact on clinical practice in the foreseeable future?

- Topical use of peptides labeled with near-infrared contrast agents may improve detection of adenomas and flat & depressed lesions in the proximal colon.
- Animal model of spontaneous dysplasia can be used to assess effectiveness of novel chemoprevention agents to reduce size of colorectal lesions for future use in patients.
- Molecular imaging has potential to validate in situ cancer biomarkers, monitor clonal evolution of progenitors, characterize risk for cancer progression, and assess likelihood for response to therapy.

Fig. 1. NIR fluorescence imaging system

A) system schematic, details in text. B) 671 nm excitation is delivered from distal end of (~3 mm diameter) endoscope. C) custom polymer housing contains filter wheel to provide reflectance and fluorescence images. D) image of standard target demonstrates sub-cellular resolution of 9.8 μm (group 5, element 5, 50.8 lp/mm), highlighted by the red oval in the inset, and large FOV of 3.6 mm at imaging distance of $d = 2.5$ mm.

Fig. 2. NIR-labeled peptides

Chemical structure of A) target (QPI-Cy5.5) peptide, includes binding motif (black) labeled with Cy5.5 (red) dye via linker (blue), and B) mass spectra signature. Chemical structure of C) control (YTT-Cy5.5) peptide and D) corresponding mass spectra signature. E) Fluorescence spectra of QPI-Cy5.5 (red) and YTT-Cy5.5 (green) peptides with $\lambda_{\text{ex}} = 671$ nm excitation show peak emission near 709 nm.

Fig. 3. High resolution targeted in vivo imaging

A) Conventional white light image of colon shows spontaneous dysplasia. B) NIR reflectance image shows amorphous surface texture while C) fluorescence image distinguishes dysplasia with high resolution and contrast. Fluorescence image with D) YTT-Cy5.5 (control) peptide and E) no peptide (autofluorescence) reveals minimal signal.

Fig. 4. Individual dysplastic crypts

A) Expanded view of yellow box in Fig. 3C) reveals individual crypts, magnification 4X, and B) representative histology (H&E) confirms pathological features of dysplasia, scale bar 100 μ m.

Fig. 5. Fluorescence image analysis

Quantitative analysis shows target peptide QPI-Cy5.5 binds to murine colonic dysplasia with greater fluorescence intensity compared to that of the control peptide YTT-Cy5.5, mean value±SD of 3.42 ± 1.30 ($n = 8$) versus 1.88 ± 0.38 ($n = 9$), respectively.

Fig. 6. Peptide immunohistochemistry

The QPI-Cy5.5 peptide demonstrates minimal fluorescence staining on A) normal compared to B) dysplasia, and C) adenocarcinoma on human surgical sections. The control peptide showed minimal fluorescence staining to D) normal, E) dysplasia, and F) adenocarcinoma. G-I) Representative histology (H&E). Scale bar; A–F) 50 μm and G–I) 200 μm.