Wide-field fluorescence endoscopy and dual-axis confocal microscopy for targeted imaging of cathepsin activity in colon cancer

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Early Detection of Cancer: Colorectal Cancer

• Third most common form of cancer affecting men and women and second most deadly
• Regular screening prevents 60% of deaths, and 9/10 patients whose cancer is detected early are alive 5 years later (CDC.gov)
• Cancerous and precancerous growths are removed during screening
• After age 50, colonoscopy recommended once every 10 years (Cancer.org)
• Because screening is so infrequent, it is especially important not to miss even early lesions
• Wide-field fluorescence imaging with cancer-targeting molecular probes may catch early lesions
Technology Overview
Wide-Field Fluorescence Endoscopy for Colon Cancer Detection

DAC microscope

Wide-field fluorescence

Digitally displayed image

70 deg. field-of-view of fiberscope

Fluorescing cancerous lesions

Digitally displayed reticle indicates position of microscope’s FOV

Slide courtesy of Mike Mandella
Wide-Field Fluorescence Endoscopy for Colon Cancer Detection

Boston Scientific SpyGlass Fiberscope

- 225 Multi-Mode Illumination Fibers
- 6600 Imaging Fibers
- ~0.4 mm
- ~0.9 mm
- 231 cm
- GRIN Lens
- 70° FOV
- 125 μm
Rodent Endoscope System
Dual-Axis Confocal (DAC) Miniature Microscope

- Optical sectioning without cutting tissue
- Image stacks: hundreds of tissue sections in one 3D acquisition
- MEMS technology and miniature optics: endoscope-compatible microscope
Targeting Colon Cancer in a Mouse Model with Cathepsins
Pan Cathepsin Probe: BMV-109

- High cathepsin protease enzyme activity in inflammation- and tumor-associated macrophages
- BMV-109 targets active enzyme, covalently binds, and de-quenches to become fluorescent

Slide courtesy of Ehud Segal

Bogyo Lab
Mouse Model of Colon Cancer

• A/J mouse aged 6-8 weeks injected i.p. with 1 mg/kg azoxymethane weekly for 6 weeks
• Develops numerous polyps in the distal colon
• Recapitulates human adenoma to carcinoma sequence
• More inclined to beta catenin pathway than APC mutation pathway

Animals prepared and maintained by Gambhir Lab
Topical Staining Protocol

- Mouse anesthetized with isoflurane, and distal colon flushed with PBS enema
- BMV-109 probe administered (200 μL, 10 μM) intra-rectally using soft catheter and syringe
- Incubation for 45 min
- Unbound probe washed out with PBS enemas
- In-vivo imaging of descending colon/rectum and ex-vivo imaging of entire colon
In-Vivo Fluorescence Imaging

White Light + Color Camera

Remove Mirror + Laser On + LED Off

Laser + EMCCD (Fluorescence)
Ex-Vivo Fluorescence Imaging
Wide-Field + DAC

iPhone Fiberscope

DAC Microscope
Biomarker-Specific Fluorescence

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<tr>
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<th>Vehicle (intrarectal-topical)</th>
<th>BMV-109 (intrarectal-topical)</th>
<th>BMV-109 (intravenous)</th>
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<td>Polyp</td>
<td>Colon</td>
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- Covalently bound probe can be imaged on fluorescence gel reader to confirm cathepsin labeling
- (Example above: BMV-109 administered topically and intravenously to APC$_{Min}$ transgenic colon cancer mice)
Vision: A Multimodal Fluorescence Endoscope
Multimodal Fluorescence Imaging

DAC Microscopy: Functional Cellular Labeling

DAC Microscopy: Morphological Counterstain
Summary

• Colon cancer prevention and survival are highly dependent on effective screening
• Wide-field fluorescence endoscopy combined with targeted molecular probes offers a new mode of sensitive imaging for early detection of tumors
• Future endoscopic tools can include wide-field and microscopic fluorescence imaging to better locate and identify cancer margins
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