Pre-Cancer Detection and Diagnosis involving Fluorescence Peptides and Laser Scanning Endoscopes

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Outline

- Cancer and pre-cancer (dysplasia)
- Colon cancer example (polyps)
- Esophageal cancer and Barrett’s esophagus
- Laser-scanning endoscope development at UW
- Visualize pre-cancer with fluorescence using peptide biomarker with conjugated fluorophore
- Animal model of colon pre-cancer & imaging
- Translation to human esophageal dysplasia
- Swallowable tethered-capsule endoscope

Cancer Progression: Cell Level

> 80% of all cancers originate in the epithelial layer (typ.<0.2 mm thick), becoming deadly when cancer makes contact with blood supply
Colorectal cancer example

5-year survival data (CRC):

- 90% if localized disease – confined to wall of the bowel
- 68% if regional disease – lymph node involvement
- 10% if distant metastases are present – Ries et al., NCI, 2007.

NOTE: Pre-malignant condition in CRC is often discernable by endoscopy. What about locating high grade dysplasia in patients with Barrett’s esophagus, which is a pre-cancer condition of esophageal cancer?

Barrett’s Esophagus (BE):
Why is it Important?

- Precursor to esophageal adenocarcinoma
- Cancer with most rapidly increasing incidence in the United States
  - 350% increase between 1974 and 1994
- 5 year survival rate of esophageal cancer in symptomatic patients is 14%
- Detection of early cancer and high grade dysplasia leads to cure in > 90% of patients
- Screening technique identified as research priority by National Cancer Institute*

*NIH/NCI Esophageal Cancer Progress Review Group – 12/02

Barrett’s Esophagus: Trends


Barrett’s Esophagus and Adenocarcinoma

Normal
Barrett’s
Adenocarcinoma
Barrett’s Esophagus: Screening/Surveillance Dilemma

- If BE, then 40x increase in cancer risk
- Even with BE, then 0.5% annual cancer risk
- 6-12% BE prevalence in patients w/GERD
- For every BE patient, 20 more undiagnosed
- GERD symptoms in 7-20% US population
- Limited to >50 years then 5 to 15 M in USA

References: Rastogi et al., 2008; Falk, 2002; Hur, 2007;

Current Problem

Goal is to reduce mortality from esophageal cancer.

- Although Barrett’s is an endoscopically visible pre-cancerous condition, doctor cannot easily see the 1 in 200 who is progressing to cancer.
- Must random biopsy the region of Barrett’s esophagus.
  - Seattle Protocol, quadrant every 1 cm axial length of Barrett’s esophagus
  - Sampling error of >90%, and bloody mess (lowers compliance)
- Compare to Colon Cancer: Pre-cancerous polyps are isolated and more easily seen and removed in endoscopy as a biopsy which confirms level of dysplasia.

Goal #1: Label high-grade dysplastic regions of Barrett’s esophagus with fluorescence biomarker to make highly visible for biopsy.

Goal #2: Develop a new type of endoscope at the UW to image cost effectively.

Multi-Investigator Solution

Early pre-malignant cancer is difficult to see in vivo.

Solution Strategy

- Label dysplasia with panel of peptide biomarkers (Tom Wang, UM)
- Red-flag early cancer by conjugation with fluorescence dyes
- Integrate into cost-effective wide-field endoscopy (SFE)
- Test in animal model of CRC and translate to human (TCE)

Hypothesis: Multiple peptides can increase sensitivity/specificity of targeted cancer screening, especially with multispectral imaging.

UW Part of the Solution
Problems with Current Endoscopes

- Sedation is required for upper GI endoscopy
- Inaccessible for small lumens
- Laser diagnostics and therapies are added size, complexity, and cost
- Ultrathin scopes are:
  - Not high in resolution
  - Not highly flexible
  - Fragile
  - Expensive
  - Imaging only function

Advantage of Smaller Diameter

e.g. the human airways

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Scanning Fiber Endoscope (SFE)

Animation by Mr. Duff Hendrickson, Seattle, WA, copyright University of Washington.

Combined into a singlemode optical fiber: R-635nm, G-532nm, and B-442nm, RGB illumination.
Mechanical Resonance

\[ F_{1,2} = \frac{\pi \sqrt{E}}{16 \sqrt{\rho}} \frac{R}{L^2} \left( 1.194^2, 2.988^2, \ldots \right) \text{ [Hz]} \]

where \( \rho \) = density, \( E \) = modulus of elasticity, \( R \) = radius and \( L \) = length of solid cylindrical fiber cantilever

Cantilever length is 4.3 mm for 5 KHz (125 \( \mu \)m) and 2.4 mm for 11 KHz (80 \( \mu \)m).

1.2-mm SFE Prototype
(30 Hz imaging of 500-line color images)

70 glass optical fibers, each 50 micron OD
1.2 mm outer diameter

Comparing ultrathin endoscopes

Fiberoptic Imaging Bundle

Dot Matrix - Printer Technology - Laser

Test Target – Single Video Frame

4-4 pattern is 22.1 micron bars
Color SFE/TCE System Block Diagram

TCE System for Clinical Testing

UW Scanning Laser Endoscopes

SFE – Scanning Fiber Endoscope
TCE – Tethered Capsule Endoscope
Unsedated (low cost) Endoscopy

- Capsule (6.4x18mm) with 1.4-mm tether
- Expected to outperform larger capsule scopes
- Addition of air channel distends esophagus
- Requires a sip of water (14 subjects so far)
- Applications are GERD - GastroEsophageal Reflux Disease, Barrett’s Esophagus, Cancer

Tethered-Capsule Endoscope TCE

Screening for esophageal cancer and Barrett's esophagus

TCE sizes:
- Tether: 1.4 mm
- Capsule: 6.4x18 mm

TCE Being Swallowed

TCE being slowly pulled out
**TCE 1st Human Test Images**

- Inside the stomach
- The gastro-esophageal junction
- In the esophagus

Seibel et al., (March 2008) IEEE Trans. Biomedical Engineering

**Enhanced Spectral Imaging (ESI)**

- TCE only
- TCE Images of Cheek and Lower Esophagus
- TCE + ESI

**Tethered-Capsule Endoscope**

Seibel et al., GI Endoscopy Clinics NA, July 2008; April 2009

- Improved spatial image resolution at near tissue to 15 microns by adjusting fiber scanner and lens assembly and increasing contrast.

**TCE w/Air Channel in vitro Testing**

Sphygmomanometer squeeze bulb (Omron) can be hand pumped 3 to 5 times to produce the necessary insufflation (100 ml within 5 seconds) to:

- Distend esophagus
- Remove bubbles
- Open lower esophageal sphincter
TCE Human Testing

Double-barrel tether is equally tolerable to single 1.4 mm diameter tether (polyurethane) in single subject.

Wide-Field Fluorescence

- 1-mm center to center grid lines marked on paper
- Pink fluorescence highlighter is marking distant lines
- Red non-fluorescence highlighter marks near lines
- Fluorescence image (B-backscatter & R-fluorescence)

Tethered-Capsule Endoscope


- 1 mm distal tip diameter and <10mm rigid tip length
- Full color (RGB – 3 narrow laser bands)
- Video (30 Hz) scanner frame rate
  - 500 line images (variable up to 1000 lines)
- 70-120 degree field-of-view lens systems
- Additional capabilities demonstrated
  - Magnification, ESI, and Fluorescence imaging
  - >4 meters long shaft has been demonstrated
  - Depth enhancement (3D)
  - Laser surgery (in vitro)
- New concept: Mosaicing used to stitch together video to form a single digital record (panorama) of the lower esophagus.

SFE/TCE Performance

- 64 images from SFE capsule used to create mosaic image
Fluorescence Biomarker Development
Peptide + Fluorophore
(University of Michigan)

Peptides and Fluorophores (Dyes)

- **7-Diethylaminocoumarin-3-carboxylic acid (DEAC/Coumarin)**
  - $\lambda_{em} = 432$ nm, $\lambda_{ex} = 472$ nm (Blue)
  - Active Peptide: KCCFPAQ
  - Control Peptide: GGGAGGG

- **5-TAMRA (5-Carboxytetramethylrhodamine) (5-TAMRA)**
  - $\lambda_{em} = 535$ nm, $\lambda_{ex} = 568$ nm (Green)
  - Active Peptide: AKPGYLS
  - Control Peptide: GGGAGGG

- **CF-633 (CF-Red)**
  - $\lambda_{em} = 630$ nm, $\lambda_{ex} = 650$ nm (Red)
  - Active Peptide: LTTHYKL
  - Control Peptide: GGGAGGG

Peptide+Dye Droplet Images
100uM, 1uM, 100nM concentrations of each peptide conjugated dye

Color SFE/TCE System Block Diagram

Red = 635 nm
Green = 532 nm
B/G = 488 nm
Blue = 442 nm
Peptide selection with phage display

- combinatorial method
- recombinant DNA technique
- high complexity library
- cell surface targets
- unbiased approach
- selective, high affinity binding

Human esophagus: Li et al., Wang (2010) Gastroenterology

Advantages of peptides

- deep mucosal penetration
- high diversity $>10^9$
- rapid binding kinetics
- low immunogenicity
- easy to label
- multimers
- SAFE for use in GI tract

Validation of peptide binding

Pilot studies at University of Michigan, Tom Wang Lab

Mice
Mice genetically engineered for somatic Apc (adenomatous polyposis coli) gene inactivation under Cre regulation spontaneously develop adenomas in the distal colon (Hinoi 2007).

Peptides:
Phage display technology was used to identify short peptide sequences that bind specifically to dysplastic cells. A method of in vivo biopanning in this mouse, Resulted in three unique peptides that bind specifically to colonic dysplasia.

In vivo imaging:
Find adenomas and flush colon with tap water using Storz scope channel, Infuse colon with 100μM biomarker, wait 5 minutes, Flush, insufflate, and image with 3.5-mm rigid Storz scope (white-light) 3-mm SFE+straw with insufflation fpr single and double channel fluorescence imaging

August 2010 – SFE testing on single peptide having one of 3 different fluorescence dyes. December 2010 – SFE testing on 3 different peptides, each having blue, green, or red dye. May 2011 - March 2012: Conference presentations and journal publication.
Mouse CRC-Model In Vivo Testing

Visible Light in Endoscopic Imaging

Single Peptide+Dye Labeling
Dual Peptide+Dye Labeling

Peptide+BlueDye and same Peptide+GreenDye are binding to same Polyp – August 2010 (Miller et al., DDW 2011 Abstract accepted, oral)

Laser “bleed-through” SFE Images

A low-cost way to provide real-time overlay of fluorescence “hot spots” within conventional wide-field endoscopic imaging showing colon structure.

Multimodal imaging of both fluorescence (dysplasia) and reflectance (structure).

Peptide+Green Dye Labeling

Storz white-light reflectance

SFE

Green fluorescence

SFE w Blue Multimodal: G-fluorescence B-reflectance

Translation to Humans
TCE and SFE are equivalent in imaging.

**Multispectral Fluorescence Imaging**

Fluorescence biomarker imaging of dysplasia in the human esophagus

**RGB & Fluorescence Signal Separation**

**Human In Vivo TCE Biomarker Testing**

**GOAL**: Red flag regions of Barrett’s esophagus that are progressing to cancer.

** Modifications required for human BE screening:**

- Add laser for fluorescein dye (FITC) excitation
- Use spray catheter to inject Peptide+FITC
- Multiple peptides with FITC conjugated dye
- Take biopsy at the fluorescence “hot spots”
- Compare image-guided fluorescence biopsy verses random biopsy or other enhanced modes of endoscopy (NBI, AF, Magnification)
Barrett’s Esophagus Human Test
TCE - 30 Hz video

BE Screening of Unsedated Patients
GOAL: Cost effective screening program for Barrett’s esophagus

Cutaway view of TCE capsule
TCE Capsule size 6.4x18mm

Instead of a parallel air channel, sheathing over tether can provide a uniform spray of fluorescence biomarkers

Summary

• Red flagging of pre-cancer in vivo – main components have been demonstrated
  – Peptides+dyes (multispectral in animals)
  – Peptide+FITC in human colon (Hsiung 2008)
  – SFE instrument (Miller et al., DDW 2011, JBO 2012)

• Future translation to human esophagus
  – Peptide+dye (FITC) in human biopsy (Li 2010)
  – TCE instrument (Dominitz & Seibel, DDW 2011)

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