Biomarkers and molecular imaging in gastrointestinal cancers

Wa Xian, Frank McKeon, Khek Yu Ho

PII: S1542-3565(13)01235-4
DOI: 10.1016/j.cgh.2013.08.033
Reference: YJCGH 53477

To appear in: Clinical Gastroenterology and Hepatology
Accepted Date: 11 August 2013

Please cite this article as: Xian W, McKeon F, Ho KY, Biomarkers and molecular imaging in gastrointestinal cancers, Clinical Gastroenterology and Hepatology (2013), doi: 10.1016/j.cgh.2013.08.033.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

All studies published in Clinical Gastroenterology and Hepatology are embargoed until 3PM ET of the day they are published as corrected proofs on-line. Studies cannot be publicized as accepted manuscripts or uncorrected proofs.
Biomarkers and molecular imaging in gastrointestinal cancers

Wa Xian¹²³⁴, Frank McKeon¹²³⁴, and Khek Yu Ho¹

¹Department of Medicine, National University Health System, Singapore; ²The Jackson Laboratory for Genomic Medicine, Farmington, CT USA; ³Genome Institute of Singapore, A-STAR, Singapore; ⁴Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT

Abbreviations:
CLE: columnar lined epithelium

Correspondence:
Khek Yu Ho, MD, FRACP, FRCP, FAMS
Department of Medicine
National University Health System, Singapore
NUHS Tower Block, Level 10
1E Kent Ridge Road,
Singapore 119228

Ph. +65 67724362
Fax: +65 67724361
E-mail: khek_yu_ho@nuhs.edu.sg

Short Title: Biomarkers and molecular imaging in gastrointestinal cancers
Word Count: 2225 words
Disclosures: The author discloses no conflicts.

Author Contributions: Frank Mc Keon and Wa Xian drafted the manuscript, and Khek–Yu Ho provided the outline and critical revision of the manuscript.
ABSTRACT

The best means to improve gastrointestinal cancer survival is screening and treatment of the early lesions. In esophageal adenocarcinoma, there is increasing thought that low-grade dysplasia and perhaps even “high-risk” Barrett’s esophagus represent the most attractive targets for achieving a cure. An issue with Barrett’s esophagus is that endoscopy alone cannot distinguish Barrett’s esophagus from columnar lined epithelium or from areas of low-grade dysplasia. Much effort has therefore been devoted to discover molecular biomarkers of “high-risk” states and develop imaging tools for detecting these biomarkers in a manner that could assist the real-time in-vivo targeting of sites for biopsy. The strategy we have employed is to generate stem cell clones from Barrett’s esophagus biopsies and compare their gene expression profiles with patient-matched stem cell clones of the esophageal squamous epithelia and gastric cardia. It is anticipated that by mining the expression datasets of these Barrett’s stem cell clones, we will be able to identify unique cell surface markers of the Barrett’s stem cells against which cytotoxic antibodies or aptamers can be developed, and used to aid the endoscopist in identifying regions of atypia for biopsy, making real-time diagnosis, stratifying patients during the examination, and ultimately directing therapy in a preemptive manner.

This review will focus on Barrett’s esophagus and its associated neoplasia to illustrate the utility of biomarker and molecular imaging in aiding targeted biopsy, making real-time diagnosis, stratifying lesions during examination, and directing treatment of this gastrointestinal disorder during surveillance endoscopy.
Gastrointestinal cancer screening to improve survival: challenges of current diagnostics and therapeutics

One of the most sobering statistics in the realm of esophageal adenocarcinoma is that an estimated 90% of cases present without prior suspicion of gastroesophageal reflux disease and therefore without prior screening.\textsuperscript{1, 2} If indeed esophageal adenocarcinoma follows the temporal progression described for other epithelial cancers,\textsuperscript{3, 4} we need to develop fundamentally new approaches to screening the at-risk patient population for treatable lesions.

With Pap-smears representing a successful model, efforts to recover cells from the distal esophagus are already undergoing clinical trials. One technology is the so-called CytoSponge, a capsule containing a sponge-like material suspended from a string is swallowed to pass the gastroesophageal junction where it expands and can be drawn back through the distal esophagus along with sheared tissue.\textsuperscript{5} While an exciting concept, hurdles remain for this and other tissue recovery approaches. For one, the cytologic analysis of recovered cells will be more complex than histologic analysis of standard biopsies obtained with the aid of endoscopy. Low-grade dysplasia, an ongoing challenge in standard distal esophageal biopsies, will be beyond the capacity of CytoSponge-recovered cells that lack the polarity cues provided by intact tissue. While a diagnosis of high-grade dysplasia triggers ablative therapies including endoscopic submucosal dissection where complication rates are high in inexperienced hands, or newer approaches such as radiofrequency ablation where reliable rates remain uncertain, there is increasing thought that low-grade dysplasia and perhaps even “high-risk” Barrett’s esophagus represent the most attractive targets for achieving a cure with minimal therapeutic morbidity.
If detecting “high-risk” Barrett’s esophagus and low-grade dysplasia are indeed the means of improving survival, it is likely that we need to invoke technologies beyond standard histology which has proven insufficient to make such calls. Several groups have shown that biopsies from Barrett’s epithelia nearby esophageal adenocarcinoma share the precise nonsynonymous mutations observed in tumor sequenced from the same patient, suggesting a role for genetic tools in stratifying risk in patients and identifying those in need of timely intervention.\textsuperscript{6,7} While the apparent molecular heterogeneity of esophageal adenocarcinoma \textsuperscript{8-10} precludes obvious signatures even for tumors at this time, there is increasing data for genetic alterations accompanying the progression to dysplasia including stabilizing p53 mutations, ploidy, and p14/p16 deletions \textsuperscript{11-12}. With the favorable trends in genomics cost structure it is likely that mutational status of tissues recovered in any format will be assessable in the near future.

**Biomarkers and molecular imaging are more accurate than endoscopy, and provide immediate data on early cancer**

A particularly vexing problem with Barrett’s esophagus is that endoscopy alone cannot distinguish Barrett’s esophagus from columnar lined epithelium (CLE) or from areas of low-grade dysplasia. This necessitates biopsy protocols that are in the end imperfect as well as delayed pathology analyses requiring later follow-up. A clear diagnosis of CLE with low risk for progressing to dysplasia requires biopsies from the neosquamocolumnar junction to rule out goblet cells typical of Barrett’s esophagus.\textsuperscript{13} Dysplasia, especially low-grade dysplasia, is often exceedingly difficult to diagnose with any certainty as the endoscopic features are open
to interpretation. Newer technologies to help target biopsy have been developed, and these include confocal laser endomicroscopy, Raman spectroscopic probe, and biomarker enhanced molecular imaging technology.

While confocal laser targeted biopsy is able to improve the endoscopic diagnostic yield, much experience is required for its effective use.\textsuperscript{14} On the other hand, Raman spectroscopic probe with its real-time software, and highly accurate diagnostic algorithm, obviates the need for subjective interpretation.\textsuperscript{15} It is a promising tool to make real-time distinction between Barrett’s esophagus, CLE, and dysplasia. Much effort has also been devoted to defining molecular changes linked to the conversion to dysplasia and developing imaging procedures for detecting these changes. One direction has been using fluorescent lectins to interrogate changes in glycosylation that might accompany the conversion to dysplasia in a manner that could assist the real-time targeting of sites for biopsy. One of these lectin conjugates, Aspergillus oryzae lectin, is showing promise though its potential as a biomarker to identify dysplastic regions needs to be validated in large studies across multiple centers.\textsuperscript{16} Despite the advent of laser capture microdissection, there is a surprising dearth of gene expression information from patient-matched samples that might distinguish Barrett’s esophagus from dysplasia. These datasets might readily aid in the identification of a set of genes that uniquely define the dysplastic regions in a sea of Barrett’s esophagus. The concepts of dysplasia across the cancer landscape are in flux but it is likely that most true dysplasia describes cells that are both immortalized and transformed as defined by in vitro models and therefore likely to have an advanced mutational profile similar to cancers.

In some initial genomic studies we are getting hints that Barrett’s esophagus may already have some of the mutations seen in esophageal adenocarcinoma, though the extent of
these as well as the presence of other genomic alterations detected in cancers remains to be determined.\textsuperscript{6,7} This matches with theoretical analyses of somatic mutations in human cancers that suggest that many are age-related somatic variations that predate the acquisition of the tumor phenotype.\textsuperscript{17} Thus we should not be surprised that “high-risk” Barrett’s esophagus, with or without the presence of low-grade dysplasia, in fact has significant numbers of nonsynonymous mutations and other genomic alterations. How these affect the physiology and gene expression of such high-risk cases is uncertain at present and needs to be assessed empirically to determine if markers of such altered states can be identified.

The strategy we have employed to address the stratification problem is to generate stem cell clones from Barrett’s esophagus biopsies and compare their gene expression profiles with patient-matched stem cell clones of the esophageal squamous epithelia and gastric cardia (Ho, McKeon, and Xian, unpublished). Of the first five series of patient-matched stem cells, we have found Barrett’s stem cells of two patients have considerably higher numbers of nonsynonymous mutations than the other three despite the absence of obvious dysplasia in biopsy specimens. The advantage of cloning stem cells of these various tissues is that we can develop pure populations of cells that represent ideal substrates for genomic analysis. This contrasts with typical Barrett’s biopsies where Barrett’s glands are intermixed with squamous islands and stromal cells such that the “Barrett’s” components might typically be present at less than 10% of the sample. We are presently mining the expression datasets of these Barrett’s stem cell clones to determine if links exist between Barrett’s esophagus with high numbers of nonsynonymous mutations and particular cell surface proteins against which antibodies can be used to aid the endoscopist in identifying regions of atypia for biopsy as well as ultimately stratifying patients during the examination.
Ironically, despite advances in stem cell cloning of epithelial tissues, cultivating dysplastic or tumor cells from human epithelial cancers has proven remarkably difficult. We are presently developing technologies to capture clones of both dysplasia and tumors to produce patient-matched series to complement those of normal and Barrett's epithelia to identify proteins that report each of these stages. It is anticipated that such markers could help in the diagnosis, risk-stratification, and ultimately the targeted therapy in efforts towards preemptive therapies, perhaps, in conjunction with more generally directed ablative therapies.

Efforts towards preemptive therapies

The poor response of upper gastrointestinal cancers such as esophageal, gastric, and pancreatic adenocarcinoma has triggered the search for preemptive therapies targeting earlier and presumably more tractable lesions. For the distal esophagus and gastric adenocarcinomas, both of which start with intestinal metaplasia and progress through dysplasia to adenocarcinoma, the targets are reasonably well defined. For Barrett's epithelium and especially Barrett's epithelium with dysplasia, the standard-of-care is rapidly becoming ablation by radiofrequency ablation. While this technology is in its infancy, it has already offered significant hope that preemptive therapies are a viable alternative to awaiting the onset of aggressive cancers. However, there is much discussion of cases of recurrence of Barrett’s epithelium, dysplasia, and even adenocarcinoma post ablation, and only time will tell us what the rate of failure in the use of nonspecific ablation therapies is in the long run.
Given that Barrett’s glands are regenerated by a unique stem cell, the likely culprit in cases of recurrence post ablation is the Barrett’s stem cell or analogous cells in Barrett’s dysplasia. Therefore such cells need to be targeted either along or together with the non-specific modalities. It follows that an important goal for the field is to identify unique cell surface markers of the Barrett’s stem cells against which cytotoxic antibodies or aptamers can be developed. Peptides selected for detection of colonic dysplasia have already been developed and shown to be highly effective approach to the general problem of targeting abnormal cells in an in vivo setting. Similar approaches to Barrett’s esophagus its associated dysplasia are some of the most promising technologies that combine detection, diagnosis, and ultimately therapeutics for the massive problem of Barrett’s disease.
References


Table: Take Home Messages

- Molecular marker assisted imaging platforms are useful for detection, diagnosis, and characterization of lesions.
- Barrett’s stem cell surface proteins can be used to develop antibodies and aptamers for diagnosis, risk stratification, and elimination of esophageal cancer precursors.