Biomarkers and Molecular Imaging in Gastrointestinal Cancers

Wa Xian,[*,‡,§,||] Frank McKeon,[*,‡,§,||] and Khek Yu Ho[*]

*Department of Medicine, National University Health System, Singapore; ‡The Jackson Laboratory for Genomic Medicine, Farmington, Connecticut; §Genome Institute of Singapore, A-STAR, Singapore; and ||Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, Connecticut

The best means to improve gastrointestinal cancer survival is screening and treatment of early lesions. In esophageal adenocarcinoma, it is believed 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, therefore, has been devoted to discover molecular biomarkers of high-risk states and to develop imaging tools for detecting these biomarkers in a manner that could assist real-time in vivo targeting of sites for biopsy. The strategy we have used is to generate stem cell clones from Barrett's esophagus biopsy specimens and to 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 data sets 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, perform a real-time diagnosis, stratify patients during the examination, and, ultimately, direct therapy in a preemptive manner.

Keywords: Barrett's Esophagus; Stratifying Biomarkers; Preemptive Therapy; Early Detection; Barrett's Stem Cells.

This review focuses on Barrett's esophagus and its associated neoplasia to illustrate the utility of biomarker and molecular imaging in aiding in targeted biopsy, making a real-time diagnosis, stratifying lesions during an 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.[1,2] If, indeed, esophageal adenocarcinoma follows the temporal progression described for other epithelial cancers,[3,4] we need to develop fundamentally new approaches to screening the at-risk patient population for treatable lesions.

With Papanicolaou smears representing a successful model, efforts to recover cells from the distal esophagus already are undergoing clinical trials. One technology is the so-called CytoSponge (Medical Research Council, Cambridge, UK), 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.[5] Although 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 biopsy specimens obtained by endoscopy. Assessing low-grade dysplasia, an ongoing challenge in standard distal esophageal biopsy specimens, will be more complex with CytoSponge-recovered cells that lack the polarity cues provided by intact tissue. Although a diagnosis of high-grade dysplasia triggers ablative therapies including endoscopic submucosal dissection, for which complication rates are high in inexperienced hands, or newer approaches such as radiofrequency ablation, in which 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 biopsy specimens from Barrett's epithelia near 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.[6,7] Although the apparent molecular heterogeneity of esophageal adenocarcinoma[8–10] precludes obvious signatures even for tumors at this

Abbreviation used in this paper: CLE, columnar-lined epithelium.

1542-3565/$36.00
http://dx.doi.org/10.1016/j.cgh.2013.08.033

time, the data are accumulating for genetic alterations accompanying the progression to dysplasia including stabilizing p53 mutations, ploidy, and the p14/p16 deletions.[11,12] With the favorable trends in genomics cost structure, it is likely that the 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 imperfect, as well as delayed pathology analyses requiring later follow-up evaluation. A clear diagnosis of CLE with low risk for progressing to dysplasia requires biopsy specimens from the neosquamocolumnar junction to rule out goblet cells typical of Barrett's esophagus.[13] Dysplasia, especially low-grade dysplasia, often is exceedingly difficult to diagnose with any certainty because the endoscopic features are open to interpretation. Newer technologies to help target biopsy have been developed, and these include confocal laser endomicroscopy, the Raman spectroscopic probe, and biomarker-enhanced molecular imaging technology.

Although confocal laser–targeted biopsy is able to improve the endoscopic diagnostic yield, much experience is required for its effective use.[14] On the other hand, the Raman spectroscopic probe with its real-time software, and highly accurate diagnostic algorithm, obviates the need for subjective interpretation.[15] It is a promising tool to make a real-time distinction between Barrett's esophagus, CLE, and dysplasia. Much effort also has been devoted to defining molecular changes linked to the conversion to dysplasia and developing imaging procedures for detecting these changes. For instance, the patterns of glycosylation of cell surface proteins might be altered upon cellular transformation, and these patterns, revealed by the binding of various lectins, could aid the endoscopist to identify early dysplastic lesions. One of these lectin conjugates, *Aspergillus oryzae* lectin, is showing promise, although its potential as a biomarker to identify dysplastic regions needs to be validated in large studies across multiple centers.[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 data sets might readily aid in the identification of a group of genes that uniquely defines 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 already may have some of the mutations seen in esophageal adenocarcinoma, although the extent of these, as well as the presence of other genomic alterations detected in cancers, remains to be determined.[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.[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 used to address the stratification problem is to generate stem cell clones from Barrett's esophagus biopsy specimens and compare their gene expression profiles with patient-matched stem cell clones of the esophageal squamous epithelia and gastric cardia (Ho et al, unpublished data). Of the first 5 series of patient-matched stem cells, we have found Barrett's stem cells from 2 patients that have considerably higher numbers of nonsynonymous mutations than the other 3 patients 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 biopsy specimens in which Barrett's glands are intermixed with squamous islands and stromal cells such that the Barrett's components typically might be present in less than 10% of the sample. We are presently mining the expression data sets 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,[18,19] 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 toward preemptive therapies, perhaps in conjunction with more generally directed ablative therapies.[20,21]