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Biomarkers in Barrett's Esophagus

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Abstract

Biomarkers are substances that can be used to indicate normal or diseased states. In clinical settings, the term biomarker generally refers to a molecular marker produced by tissues whose detection heralds a diseased state. For patients with Barrett's esophagus, there are at least three clinical settings in which the use of molecular biomarkers has been proposed including 1) stratifying the risk of neoplastic progression, 2) serving as an adjunct to aid in the diagnosis of dysplasia, and 3) predicting response to ablative therapies. Although the routine clinical use of biomarkers in any of these clinical settings is not yet recommended, it seems reasonable to assume that biomarker validation studies will be carried out in the coming years and that movement into the clinics will be inevitable. This article reviews the current progress in using biomarkers in each of the clinical settings described above with a focus on the molecular biomarkers which have advanced the farthest toward use in routine clinical practice.

Introduction

In Barrett's esophagus, the use of molecular biomarkers has been proposed 1) to stratify the risk of neoplastic progression, 2) as an adjunct to aid in the diagnosis of dysplasia, and 3) to predict response to ablative therapies. In general the proposed molecular biomarkers fall into broad sweeping categories which represent properties of malignant cells that must be acquired by benign cells as cancer ensues. These properties include independence from exogenous mitogenic stimulation, resistance to growth-inhibitory signals, avoidance of apoptosis, limitless replication, development of adequate vascular supplies, the capacity to invade and metastasize, and an inflammatory microenvironment (Table 1).¹ To increase the efficiency of benign cells acquiring the numerous genetic alterations necessary to acquire these properties of cancer, the genomes of the cells also must become unstable. This instability is reflected by the presence of aneuploidy. This article reviews the process for bringing potential biomarkers into routine clinical practice, current progress in using biomarkers in each of the clinical settings described above with a focus on the molecular biomarkers which have advanced the farthest toward use in routine clinical practice, and genome wide assays that have the potential for both biomarker discovery and for serving as potential biomarker panels themselves.

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Validation of Biomarkers-Getting from the Bench to the Bedside

The National Cancer Institute established the Early Detection Research Network (EDRN) to support collaborative efforts in order to facilitate the movement of biomarkers to clinical validation.² The EDRN has proposed 5 phases of study that biomarkers must undergo for validation (Figure 1).^{2,3} Phase 1 is the Preclinical Exploratory Phase in which a potential biomarker is initially identified (i.e. the bench). In Phase 2, the Clinical Assay and Validation Phase, the clinical assay to detect the biomarker is developed and then tested to see if the biomarker assay can distinguish among individuals with and without the disease. Phase 3 is the Retrospective Longitudinal Phase in which the biomarker assay is used on stored tissues that were collected prospectively to determine the ability of the biomarker to detect the disease prior to clinical presentation. In Phase 4, the Prospective Screening Phase, the biomarker assay is now applied prospectively to individuals and the characteristics of the disease detected are noted as well as the false rate of disease detection. Finally, in Phase 5, the Cancer Control Phase, the biomarker is used in a large population to evaluate its ability to detect the disease and determine the overall impact on the population by screening for the biomarker. The majority of biomarkers discussed below for Barrett's esophagus have been evaluated in Phase 3 studies and none have yet been evaluated in either Phase 4 or 5 studies. Among the reasons that the movement of biomarkers from discovery to clinical validation has been hindered are that data on biomarkers generated from one research laboratory are often not reproducible in another laboratory and that the assays used to detect the biomarker in an individual lab are not generalizable. Unfortunately, such issues have hindered the clinical validation of biomarkers for Barrett's esophagus. On the bright side, the last few years have seen the collaboration of investigators at multiple institutions implementing Phase 3 biomarker studies.^{4,5} Thus it is reasonable to assume that further biomarker validation studies will be carried out in the coming years.

Molecular Biomarkers to Stratify the Risk of Neoplastic Progression

The majority of the biomarkers that have been proposed for use to risk-stratify patients with Barrett's esophagus have been shown to be predictive of cancer progression in cross-sectional studies only and none have yet been evaluated in controlled, prospective clinical trials. However, there are a few more promising biomarkers whose predictive abilities have been validated in large studies in which the tissue specimens were collected from patients in a prospective fashion, but the biomarker analyses of these tissue specimens were performed retrospectively (Phase 3). These more promising individual biomarkers include aneuploidy/tetraploidy and 17p loss of heterozygosity (LOH), as well as a few promising biomarker panels.

Aneuploidy/Tetraploidy

Genomic instability can manifest as abnormalities in chromosomal content due to losses or gains in whole chromosomes or in segments of chromosomes. Aneuploidy indicates a cell whose chromosomal content is abnormal. Aneuploidy can be detected in fresh frozen tissues by flow cytometry or in esophageal brushings and paraffin tissues by fluorescence in situ-hybridization (FISH). A number of studies have shown that aneuploidy and/or increased tetraploidy (tetraploid 4N fractions >6%) detected by flow cytometry can predict neoplastic progression of Barrett's esophagus perhaps even more accurately than the histologic grade of dysplasia.⁶⁻⁸ In one study, for example, the 5 year cumulative cancer incidence in patients whose biopsies demonstrated no dysplasia, indefinite, or low grade dysplasia was found to be 4% (95% CI, 1.6-9.0) whereas in those patients whose biopsies demonstrated high grade dysplasia the incidence of cancer was 59% (95% CI, 44.0-74.0).⁶ Thus the relative risk for cancer progression in biopsies demonstrating high grade dysplasia compared to those with lesser grades of dysplasia was 28% (95% CI, 13.0-63.0)(6). The finding of

either aneuploidy or increased tetraploidy in biopsies demonstrating high grade dysplasia added very little to the predictive strength of a histologic finding of high grade dysplasia.^{6,7} In biopsies with no dysplasia, indefinite, or low grade dysplasia that lacked aneuploidy or increased tetraploidy, the 5-year cancer incidence was found to be 0% (95% CI, 0–4.7). However, if biopsies containing these same grades of dysplasia demonstrated either aneuploidy or increased tetraploidy, the 5-year risk of cancer progression was 28% (95% CI, 12.0–55.0) (Figure 2). Thus the finding of aneuploidy or tetraploidy carried a relative risk of cancer progression of 19 (95% CI, 4.7–78.0) in patients whose biopsies demonstrated no dysplasia, indefinite, or low grade dysplasia.⁶ These findings have been subsequently confirmed in another study by these same investigators.⁷ Thus, biomarkers may eventually be most helpful in stratifying cancer risk in the subgroup of patients demonstrating no dysplasia, indefinite, or low grade dysplasia. In these studies, aneuploidy and increased tetraploidy were detected by flow cytometry of fresh, frozen tissue specimens. However this technique is not widely available for routine clinical use; which has hindered movement of these biomarkers into clinical practice. In small studies, these same biomarkers have been shown to predict neoplastic progression of Barrett's esophagus when detected using automated image cytometry and FISH, techniques which are more feasible for routine clinical practice.^{9,10}

17pLOH

Loss of heterozygosity (LOH) refers to the loss of a section of one of the two alleles which comprise a chromosome. Within this lost chromosomal segment are a number of different genes. For example, the p53 gene is located on the small arm (p) of chromosome 17 and 17pLOH refers to loss of this small arm from one allele of the chromosome and thus loss of one copy of the p53 gene. In Barrett's esophagus, the finding of 17pLOH has shown utility as an individual biomarker to predict cancer progression in Barrett's esophagus. In Barrett's patients whose biopsies contained grades of dysplasia ranging from no dysplasia to high grade dysplasia, the 3-year cumulative incidence of cancer was 38% (95% CI, 26.0–54.0) in those biopsies with 17pLOH compared to 3.3% (95% CI, 1.4–8.0) in those biopsies which retained both alleles of 17p (Figure 3).¹¹ In the subset of patients whose biopsies demonstrated no dysplasia, indefinite, or low grade dysplasia, 17pLOH was a significant predictor of progression to high grade dysplasia (RR=3.6, 95% CI, 1.3–10).¹¹ Even in those patients with high grade dysplasia, 17pLOH remained a significant predictor of progression to cancer at 3 years (RR=3.0, 95% CI, 1.1–8.2).¹¹ Other studies by this same group confirmed these findings.¹² In these studies, 17pLOH was detected in flow cytometry-purified cells, isolated from fresh frozen tissues, which then underwent whole-genome amplification followed by genotypic analyses for LOH, techniques which are quite labor intensive. More recently, a number of cross sectional studies have demonstrated promising results using FISH analyses on biopsy tissues and on brush cytology specimens of Barrett's esophagus to detect 17p LOH.^{13–16} However, FISH demonstrated a lower sensitivity than the flow cytometric approach in detecting 17pLOH when these techniques were compared head-to-head.¹⁴

Biomarker Panels

Combinations of biomarkers in panels may be better at predicting the risk of neoplastic progression in patients with Barrett's esophagus than individual biomarkers alone. For example, when aneuploidy/increased tetraploidy, 17pLOH, and 9pLOH, the chromosomal locus for p16, were used in combination in biopsies of Barrett's esophagus, the presence of all 3 abnormalities predicted an 80% risk of cancer at 6 years (Figure 4).¹² In contrast, Barrett's patients whose biopsies did not demonstrate any of these 3 biomarker abnormalities had an incidence of cancer at 10 years of 12%.¹² Thus the relative risk of

cancer progression for those patients whose biopsies had an abnormal biomarker panel compared to those with a normal panel was 38.7 (95% CI, 10.8–138.5).¹²

Another panel that has shown promise in predicting cancer progression is a gene methylation-based biomarker panel. The addition of methyl groups to the promoter regions of genes can lead to gene silencing. Methylation of the genes p16, RUNX3, or HPP1 in biopsies of Barrett's esophagus was associated with a significantly increased risk of progression to high grade dysplasia or cancer.¹⁷ These genes were then combined with 3 additional genes into a methylation-based biomarker panel along with the patient's age and length of Barrett's segment to generate a prediction model for risk of progression to high grade dysplasia or cancer.¹⁷ When applied in a retrospective fashion on tissues collected prospectively from a cohort of patients with Barrett's esophagus, this prediction model identified those patients who progressed to high grade dysplasia or cancer as early as two years before the diagnosis of neoplasia was made.¹⁷ A more recent study has confirmed the ability of a methylation-based biomarker panel to predict neoplastic progression in Barrett's esophagus.⁴

Molecular Biomarkers as an Adjunct in Diagnosing Dysplasia

Our current method to stratify the risk of cancer progression in patients with Barrett's esophagus is based on the histologic grade of dysplasia. However, dysplasia is an imperfect predictor of cancer risk for a number of reasons including poor intra- and inter-observer reproducibility of dysplasia interpretations and a poor predictive value in finding negative, indefinite, low grade, and even high grade dysplasia.^{18–20} In fact, only approximately 30% of patients with high grade dysplasia will develop cancer within 5 years.²¹ Thus molecular biomarkers have been evaluated for their ability to aid in the detection of dysplasia and in differentiating dysplasia from reactive epithelial changes caused by reflux esophagitis.²²

p53 immunostaining has been extensively studied as an adjunct to the diagnosis of dysplasia. However its use still remains quite controversial due to factors such as high rates of staining in non-dysplastic Barrett's biopsies, high rates of false positive staining, and variability in staining depending on the specific type of p53 antibody used.²² Immunostaining for alpha-methylacyl-CoA racemase (AMACR) or for a panel of biomarkers including β -catenin, cyclin D1 and p53 has been found to be useful as an adjunct in establishing the diagnoses of low grade and high grade dysplasia in Barrett's esophagus.^{23, 24} Perhaps of even more utility is the potential for these biomarkers to distinguish atypia related to epithelial cell regeneration which can often be mistaken for low grade dysplasia.^{23–25} In contrast to the promising results using the technique of immunostaining, detection of biomarkers by mRNA expression levels appears to be limited by significant inter- and intra-patient variations in gene expression levels.²⁶

Molecular Biomarkers to Predict Response to Ablative Therapy

Recent studies have shown that endoscopic ablation and endoscopic mucosal resection can prevent the development of cancer for patients with high-grade dysplasia in Barrett's esophagus.^{27–29} A number of studies have shown promise in using biomarkers including p16 loss, p53, Ki-67 (a proliferation marker), and aneuploidy for stratifying the risk of progression to high grade dysplasia or cancer in recurrent and/or residual Barrett's mucosa following ablative therapies.^{30–33} In contrast, p53 and Ki-67 immunostains of cancers removed by circumferential endoscopic mucosal resection were not useful in predicting which patients would develop recurrent cancers.³⁴ Biomarkers have also been investigated for their utility in predicting recurrence of non-dysplastic Barrett's epithelium following ablative therapies. In small cross-sectional studies on the use of ablation for reversal of non-dysplastic Barrett's epithelium, post-ablation neo-squamous epithelium demonstrated the

same biomarker abnormalities detected in the pre-ablation Barrett's epithelium suggesting that Barrett's mucosa may recur in the neo-squamous epithelium.^{35, 36} Finally, biomarkers may also be useful to select a subgroup of Barrett's patients that are likely to respond to ablative therapies. For example, one large prospective study found that loss of p16 in the Barrett's epithelium from those patients who had high-grade dysplasia or intramucosal carcinoma predicted a poor response to photodynamic therapy at 3 months (i.e. persistent dysplasia).³⁷

Genome Wide Approaches to Molecular Biomarker Discovery

In recent years, genome wide approaches have been undertaken to identify novel genes involved in human disease. For example, genome wide profiling can be done using single nucleotide polymorphism (SNP) arrays, DNA methylation profiling, mRNA microarrays, microRNA (miRNA) arrays, or protein expression arrays. Among individuals, the human genome contains millions of DNA sequence variations for the same gene and each different sequence variation is termed an allele.³⁸ One of the most common forms of sequence variation is the SNP, in which one, single nucleotide at a certain location within a gene, differs among individuals.³⁸ SNP arrays allow investigators to scan the genome for particular SNPs that appear to be more commonly associated with individuals who have the disease under study compared to healthy controls. For example, SNP analyses of tissue samples of Barrett's-associated adenocarcinoma demonstrate alterations in DNA copy number (gains or losses) of a large number of SNP regions compared to normal squamous tissues and the frequency of SNP alterations has been found to increase as the degree of dysplasia increases.^{39–41} A genome wide approach has also been taken to profile alterations in DNA methylation. Unlike SNPs which are changes in the sequence of the genome, DNA methylation is an epigenetic event. Epigenetic events change gene expression levels by mechanisms other than altering the DNA sequence. In the case of methylation, the gene expression is changed by the addition of methyl groups to cytosines that reside in CpG rich regions called "CpG islands".⁴² As noted above, the ability of methylation-based arrays has shown promise in a number of studies in predicting neoplastic progression in Barrett's esophagus.⁴ In contrast to SNPs and methylation profiling which determine alterations at the level of genomic DNA, mRNA microarrays assess changes in mRNA expression. A number of cross sectional studies have demonstrated the ability to distinguish between tissue samples of normal esophageal squamous epithelium, metaplastic Barrett's epithelium, and esophageal adenocarcinoma solely on the expression levels of numerous mRNAs on microarrays.^{43, 44} Another type of RNAs used on arrays is miRNAs. miRNAs are small, naturally occurring RNAs that inactivate multiple target genes by destabilizing that target genes mRNA or preventing the translation of that RNA into protein.⁴⁵ A few studies have identified specific miRNAs that may play key roles in Barrett's carcinogenesis which might serve as potential biomarkers.^{46, 47} Moreover in one study by Feber et al., miRNA expression patterns were shown to discriminate between benign and malignant Barrett's tissues better than mRNA expression arrays supporting their possible use as a biomarker panel.⁴⁸ Finally, proteomics research focuses on identifying differentially expressed proteins within samples. In this approach, proteins are separated from each other either by "two dimensional" gel electrophoresis in which proteins are separated based on charge, the first dimension, followed by separation based on molecular weight, the second dimension.⁴⁹ Once the proteins are separated, mass spectrometry is used for their sequence identification.⁴⁹ A number of studies have identified proteins differentially expressed between tissue samples of normal esophagus, Barrett's metaplasia, and esophageal adenocarcinoma.^{50–52} Perhaps we will see in the future combinations of these differentially expressed protein biomarkers on arrays for a global analysis of altered protein expression during the neoplastic progression of Barrett's esophagus.

Summary

The use of molecular biomarkers has been proposed 1) to stratify the risk of neoplastic progression, 2) as an adjunct to aid in the diagnosis of dysplasia, and 3) to predict response to ablative therapies. The best evidence exists for the utility of biomarkers either individually or in biomarker panels to risk stratify neoplastic progression in patients with Barrett's esophagus. Currently, however, none of these biomarkers have been validated in controlled, prospective clinical trials (Phase 4 or 5). Preliminary studies using immunohistochemical techniques to detect biomarkers show the most promise as adjuncts to aid in distinguishing reactive atypia from dysplasia in Barrett's esophagus, however their use still requires further testing and validation. Overall, far more data are needed before the use of biomarkers can be recommended as (1) predictors of post-ablation recurrence of Barrett's epithelium, Barrett's with dysplasia or cancer or (2) a means to select Barrett's patients more likely to respond to ablative therapies. Thus, the routine clinical use of biomarkers in any of these clinical settings is not yet recommended. Given the recent progress of multi-institutional collaborative studies on biomarker validation, it seems reasonable to assume that the movement of biomarkers into the clinics for widespread use will be inevitable.

Acknowledgments

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Reference List

- Colotta F, Allavena P, Sica A, et al. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis* 2009;30:1073–1081. [PubMed: 19468060]
- Srivastava S. Cancer biomarker discovery and development in gastrointestinal cancers: early detection research network-a collaborative approach. *Gastrointest Cancer Res* 2007;1:S60–S63. [PubMed: 19360150]
- Sullivan PM, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001;93:1054–1061. [PubMed: 11459866]
- Jin Z, Cheng Y, Gu W, et al. A multicenter, double-blinded validation study of methylation biomarkers for progression prediction in Barrett's esophagus. *Cancer Res* 2009;69:4112–4115. [PubMed: 19435894]
- Sato F, Jin Z, Schulmann K, Wang J, et al. Three-tiered risk stratification model to predict progression in Barrett's esophagus using epigenetic and clinical features. *PLoS One* 2008;3:e1890. [PubMed: 18382671]
- Reid BJ, Levine DS, Longton G, et al. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. *Am J Gastroenterol* 2000;95:1669–1676. [PubMed: 10925966]
- Rabinovitch PS, Longton G, Blount PL, et al. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. *Am J Gastroenterol* 2001;96:3071–3083. [PubMed: 11721752]
- Kerkhof M, Steyerberg EW, Kusters JG, et al. Aneuploidy and high expression of p53 and Ki67 is associated with neoplastic progression in Barrett esophagus. *Cancer Biomark* 2008;4:1–10. [PubMed: 18334729]
- Huang Q, Yu C, Zhang X, et al. Comparison of DNA histograms by standard flow cytometry and image cytometry on sections in Barrett's adenocarcinoma. *BMC Clin Pathol* 2008;8:5. [PubMed: 18513411]
- Rygiel AM, Milano F, Ten Kate FJ, et al. Assessment of chromosomal gains as compared to DNA content changes is more useful to detect dysplasia in Barrett's esophagus brush cytology specimens. *Genes Chromosomes Cancer* 2008;47:396–404. [PubMed: 18265409]

11. Reid BJ, Prevo LJ, Galipeau PC, et al. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. *Am J Gastroenterol* 2001;96:2839–2848. [PubMed: 11693316]
12. Galipeau PC, Li X, Blount PL, et al. NSAIDs modulate CDKN2A, TP53, and DNA content risk for progression to esophageal adenocarcinoma. *PLoS Med* 2007;4:e67. [PubMed: 17326708]
13. Fahmy M, Skacel M, Gramlich TL, et al. Chromosomal gains and genomic loss of p53 and p16 genes in Barrett's esophagus detected by fluorescence in situ hybridization of cytology specimens. *Mod Pathol* 2004;17:588–596. [PubMed: 15017433]
14. Wongsurawat VJ, Finley JC, Galipeau PC, et al. Genetic mechanisms of TP53 loss of heterozygosity in Barrett's esophagus: implications for biomarker validation. *Cancer Epidemiol Biomarkers Prev* 2006;15:509–516. [PubMed: 16537709]
15. Cestari R, Villanacci V, Rossi E, et al. Fluorescence in situ hybridization to evaluate dysplasia in Barrett's esophagus: a pilot study. *Cancer Lett* 2007;251:278–287. [PubMed: 17321043]
16. Rygiel AM, van Baal JW, Milano F, et al. Efficient automated assessment of genetic abnormalities detected by fluorescence in situ hybridization on brush cytology in a Barrett esophagus surveillance population. *Cancer* 2007;109:1980–1988. [PubMed: 17385213]
17. Schulmann K, Sterian A, Berki A, et al. Inactivation of p16, RUNX3, and HPP1 occurs early in Barrett's-associated neoplastic progression and predicts progression risk. *Oncogene* 2005;24:4138–4148. [PubMed: 15824739]
18. Reid BJ, Haggitt RC, Rubin CE, et al. Observer variation in the diagnosis of dysplasia in Barrett's esophagus. *Hum Pathol* 1988;19:166–178. [PubMed: 3343032]
19. Montgomery E, Bronner MP, Goldblum JR, et al. Reproducibility of the diagnosis of dysplasia in Barrett esophagus: a reaffirmation. *Hum Pathol* 2001;32:368–378. [PubMed: 11331953]
20. Downs-Kelly E, Mendelin JE, Bennett AE, et al. Poor interobserver agreement in the distinction of high-grade dysplasia and adenocarcinoma in pretreatment Barrett's esophagus biopsies. *Am J Gastroenterol* 2008;103:2333–2340. [PubMed: 18671819]
21. Spechler SJ. Dysplasia in Barrett's esophagus: limitations of current management strategies. *Am J Gastroenterol* 2005;100:927–935. [PubMed: 15784042]
22. Odze RD. Diagnosis and grading of dysplasia in Barrett's oesophagus. *J Clin Pathol* 2006;59:1029–1038. [PubMed: 17021130]
23. van Dekken H, Hop WC, Tilanus HW, et al. Immunohistochemical evaluation of a panel of tumor cell markers during malignant progression in Barrett esophagus. *Am J Clin Pathol* 2008;130:745–753. [PubMed: 18854267]
24. Dorer R, Odze RD. AMACR immunostaining is useful in detecting dysplastic epithelium in Barrett's esophagus, ulcerative colitis, and Crohn's disease. *Am J Surg Pathol* 2006;30:871–877. [PubMed: 16819330]
25. Shi XY, Bhagwande B, Leong AS. p16, cyclin D1, Ki-67, and AMACR as markers for dysplasia in Barrett esophagus. *Appl Immunohistochem Mol Morphol* 2008;16:447–452. [PubMed: 18665038]
26. Hennig EE, Mikula M, Orlowska J, et al. Large intra- and inter-individual variability of genes expression levels limits potential predictive value of molecular diagnosis of dysplasia in Barrett's esophagus. *J Mol Med* 2008;86:233–242. [PubMed: 17952395]
27. Overholt BF, Wang KK, Burdick JS, et al. Five-year efficacy and safety of photodynamic therapy with Photofrin in Barrett's high-grade dysplasia. *Gastrointest Endosc* 2007;66:460–468. [PubMed: 17643436]
28. Shaheen NJ, Sharma P, Overholt BF, et al. Radiofrequency ablation in Barrett's esophagus with dysplasia. *N Engl J Med* 2009;360:2277–2288. [PubMed: 19474425]
29. Pech O, Behrens A, May A, et al. Long-term results and risk factor analysis for recurrence after curative endoscopic therapy in 349 patients with high-grade intraepithelial neoplasia and mucosal adenocarcinoma in Barrett's oesophagus. *Gut* 2008;57:1200–1206. [PubMed: 18460553]
30. Hage M, Siersema PD, Vissers KJ, et al. Molecular evaluation of ablative therapy of Barrett's oesophagus. *J Pathol* 2005;205:57–64. [PubMed: 15586364]

31. Hornick JL, Mino-Kenudson M, Lauwers GY, et al. Buried Barrett's epithelium following photodynamic therapy shows reduced crypt proliferation and absence of DNA content abnormalities. *Am J Gastroenterol* 2008;103:38–47. [PubMed: 18076737]
32. Dvorak K, Ramsey L, Payne CM, et al. Abnormal expression of biomarkers in incompletely ablated Barrett's esophagus. *Ann Surg* 2006;244:1031–1036. [PubMed: 17122630]
33. Prasad GA, Wang KK, Halling KC, et al. Correlation of histology with biomarker status after photodynamic therapy in Barrett esophagus. *Cancer* 2008;113:470–476. [PubMed: 18553366]
34. Lopes CV, Mnif H, Pesenti C, et al. p53 and Ki-67 in Barrett's carcinoma: is there any value to predict recurrence after circumferential endoscopic mucosal resection? *Arq Gastroenterol* 2007;44:304–308. [PubMed: 18317648]
35. Lopes CV, Pereira-Lima J, Hartmann AA. p53 immunohistochemical expression in Barrett's esophagus before and after endoscopic ablation by argon plasma coagulation. *Scand J Gastroenterol* 2005;40:259–263. [PubMed: 15932166]
36. Garewal H, Ramsey L, Sharma P, et al. Biomarker studies in reversed Barrett's esophagus. *Am J Gastroenterol* 1999;94:2829–2833. [PubMed: 10520829]
37. Prasad GA, Wang KK, Halling KC, et al. Utility of biomarkers in prediction of response to ablative therapy in Barrett's esophagus. *Gastroenterology* 2008;135:370–379. [PubMed: 18538141]
38. Christensen K, Murray JC. What genome-wide association studies can do for medicine. *N Engl J Med* 2007;356:1094–1097. [PubMed: 17360987]
39. Wiech T, Nikolopoulos E, Weis R, et al. Genome-wide analysis of genetic alterations in Barrett's adenocarcinoma using single nucleotide polymorphism arrays. *Lab Invest* 2009;89:385–397. [PubMed: 18663352]
40. Li X, Galipeau PC, Sanchez CA, et al. Single nucleotide polymorphism-based genome-wide chromosome copy change, loss of heterozygosity, and aneuploidy in Barrett's esophagus neoplastic progression. *Cancer Prev Res* 2008;1:413–423.
41. Paulson TG, Maley CC, Li X, et al. Chromosomal instability and copy number alterations in Barrett's esophagus and esophageal adenocarcinoma. *Clin Cancer Res* 2009;15:3305–3314. [PubMed: 19417022]
42. Hahn, MA.; Pfeifer, GP. *Mutat Res Epress*. 2009. Methods for genome-wide analysis of DNA methylation in intestinal tumors.
43. Selaru FM, Zou T, Xu Y, et al. Global gene expression profiling in Barrett's esophagus and esophageal cancer: a comparative analysis using cDNA microarrays. *Oncogene* 2002;21:475–478. [PubMed: 11821959]
44. Greenawalt DM, Duong C, Smyth GK, et al. Gene expression profiling of esophageal cancer: comparative analysis of Barrett's esophagus, adenocarcinoma, and squamous cell carcinoma. *Int J Cancer* 2007;120:1914–1921. [PubMed: 17236199]
45. Kan T, Meltzer SJ. MicroRNAs in Barrett's esophagus and esophageal adenocarcinoma. *Curr Opin Pharmacol* 2009;9:727–732. [PubMed: 19773200]
46. Kan T, Sato F, Ito T, et al. The miR-106b-25 polycistron, activated by genomic amplification, functions as an oncogene by suppressing p21 and Bim. *Gastroenterology* 2009;136:1689–1700. [PubMed: 19422085]
47. Maru DM, Singh RR, Hannah C, et al. MicroRNA-196a is a potential marker of progression during Barrett's metaplasia-dysplasia-invasive adenocarcinoma sequence in esophagus. *Am J Pathol* 2009;174:1940–1948. [PubMed: 19342367]
48. Feber A, Xi L, Luketich JD, et al. MicroRNA expression profiles of esophageal cancer. *J Thorac Cardiovasc Surg* 2008;135:255–260. [PubMed: 18242245]
49. Zhang DY, Ye F, Gao L, et al. Proteomics, Pathway Array and Signaling Network-Based Medicine in Cancer. *Cell Div* 4:20. Epub, 2009. [PubMed: 19863813]
50. Peng D, Sheta EA, Powell SM, et al. Alterations in Barrett's-related adenocarcinomas: a proteomic approach. *Int J Cancer* 2008;122:1303–1310. [PubMed: 18000824]
51. Zhao J, Chang AC, Li C, et al. Comparative proteomics analysis of Barrett metaplasia and esophageal adenocarcinoma using two-dimensional liquid mass mapping. *Mol Cell Proteomics* 2007;6:987–999. [PubMed: 16829691]

52. Streitz JM Jr, Madden MT, Marimanikkuppam SS, et al. Analysis of protein expression patterns in Barrett's esophagus using MALDI mass spectrometry, in search of malignancy biomarkers. *Dis Esophagus* 2005;18:170–176. [PubMed: 16045579]

Phases of Biomarker Research (National Cancer Institute EDRN)

Figure 1.

Aneuploidy and ↑ Tetraploidy May Predict Cancer Progression

Biopsies of Low Grade, Indefinite, and No Dysplasia

Figure 2.

17pLOH May Predict Cancer Progression

Figure 3.

17pLOH, DNA Content, or 9pLOH May Predict Cancer Progression

Figure 4.

Table 1

Summary of the cancer properties acquired during neoplastic progression of Barrett's esophagus and the more promising individual biomarkers proposed to risk stratify patients with Barrett's esophagus.

Cancer Hallmark	Promising Biomarker
DNA damage/Genomic Instability	Aneuploidy/↑Tetraploidy
Independence from exogenous stimulation	None
Resistance to Growth Inhibitory Signals	17pLOH (p53)
Avoidance of Apoptosis	17pLOH (p53)
Limitless Replication	None
Development of Adequate Vascular Supplies	None
Invasion and Metastasis	None
Inflammatory Microenvironment	None