Focus on Barrett's esophagus and esophageal adenocarcinoma

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Introduction

Esophageal malignancies are the sixth leading cause of cancer death in the world. Esophageal cancers represent about 1 percent of the cancers diagnosed in the US, with an estimated 14,250 cases in 2004, 75% of which will affect men (American Cancer Society, 2004). Despite its relatively low incidence, esophageal cancer is the seventh leading cause of cancer death among US men. Two major types of cancer arise in the esophagus: squamous cell carcinoma, which is associated with chronic smoking and alcohol consumption in the US, and adenocarcinoma, which typically arises in a premalignant condition called Barrett's esophagus (BE). This review will concentrate on BE and esophageal adenocarcinoma (EA). BE is a condition in which the normal squamous epithelium lining the esophagus is replaced with an intestinal metaplasia as a result of chronic gastroesophageal reflux disease (GERD) (Figure 1). BE was not definitively described until 1950 by the English surgeon Norman Barrett, and the first large series linking GERD, BE, and EA was reported by Naef in 1975. EA was considered a rare diagnosis even into the 1980s; in the past 30 years, however, the incidence of EA has risen at an alarming rate in the United States and other Western countries, for reasons that are largely unknown (Brown et al., 2002).

Major risk factors for development of EA include symptomatic GERD (heartburn), obesity, and tobacco use (Lagergren et al., 1999; Vaughan et al., 1995). Several early reports found no evidence for inherited predisposition, although a recent study has reported familial aggregation of BE, EA, and adenocarcinoma of the gastric cardia (Chak et al., 2002). Although reflux esophagitis was first described in 1935, symptomatic reflux now affects almost 20% of the adult US population on a weekly basis (Locke et al., 1997). The exact pathophysiology of BE is unknown, but it is thought that chronic exposure to acid and bile during reflux causes damage and inflammation in the esophageal squamous epithelium, and approximately 10% of GERD patients have already developed BE when they seek medical attention. Patients with BE progress to EA at a rate of approximately 0.5%–1.0% per year, although estimates of progression vary and population estimates are not available because most patients diagnosed with EA are not in surveillance programs. Affected individuals are predominantly male (about 75%), older, and Caucasian (Brown and Devesa, 2002). The incidence of EA has dramatic regional variations between and within countries. Western, industrialized nations have higher incidences of EA, with the highest rates reported in Scotland, and regional differences exist within countries; for example, in United States, the incidence of EA in Seattle, Washington is twice that of Utah or Atlanta, Georgia (Brown et al., 2002).

Despite the fact that many patients with BE experience GERD symptoms, the majority of patients who develop EA present with advanced, metastatic disease at first examination (Lagergren et al., 1999). Endoscopic examination with biopsy is required for definitive diagnosis of BE and EA. Management of BE consists of treating the underlying GERD, namely reducing reflux of stomach and duodenal contents into the esophagus, and surveillance for the early detection of EA. Medical therapies consist of medications that block the production of acid in the stomach, such as proton pump inhibitors (e.g., omeprazole). Proton pump inhibitors strongly reduce acid output and reflux volume, generally achieving excellent symptom relief, and they have become the medication of choice for most patients with severe reflux and BE. The goal of anti-reflux surgery (e.g., Hill repair, Nissan fundoplication) is to restore the normal barrier against reflux. These procedures can be effective in reducing reflux, although there is a learning curve for antireflux surgery and the reported mortality in the community is 0.8% (Flum et al., 2002). There are also some concerns over reduced efficacy of the repair over time. Many BE patients treated with medical therapy to relieve symptoms may continue to have silent reflux, and one study has suggested that only effective control of reflux can reduce the risk of developing BE (Oberg et al., 2001). While effective acid reduction may decrease proliferation in the Barrett's epithelium, this has not been proven in randomized trials, and there is no evidence that eliminating gastroesophageal reflux reduces the risk of developing EA in patients with BE. In fact, two recent studies have suggested that acid reduction therapy may increase proliferation and decrease apoptosis in BE by elevating serum gastrin (see Harris et al., 2004).

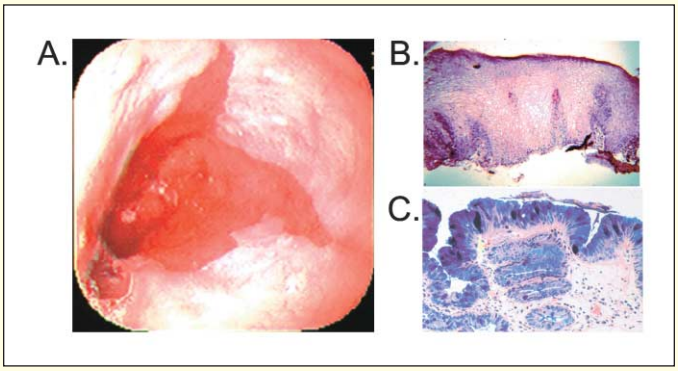

Figure 1. Barrett's esophagus

A: White light image of Barrett's intestinal metaplasia as observed during endoscopic surveillance. The red areas are the Barrett's metaplasia and the whitish pink areas are normal squamous epithelium.
B: Microscopic cross-section of esophageal squamous epithelium.
C: Microscopic section of Barrett's intestinal metaplasia. Note the crypt architecture of the Barrett's epithelium, similar to that of the small intestine, and the pathognomonic presence of goblet cells (very dark blue staining).

In spite of improvements in surgical, chemo-, and radiation therapy, the prognosis for patients presenting with advanced EA is poor, with the most recent statistics showing 5-year survival rates of 0.9% for those with distant disease (Brown and Devesa, 2002). Since most patients with BE will not develop EA, and those who do are faced with very poor prognosis unless detected early, identification of those patients at greatest risk is critical for improving survival from EA. Surgical removal of the esophagus (esophagectomy) is the only proven cure for EA localized to the esophagus. However, esophagectomy has significant morbidity and mortality dependent upon the volume of procedures performed at a given institution. Although chemotherapy and radiation may be offered in patients who have metastatic disease that cannot be removed during esophagectomy, resistance typically emerges to the chemotherapy, and the prognosis is poor. Palliation to preserve the ability to swallow becomes very important in maintaining quality of life. Although there is no universal strategy, palliation can be accomplished by varying combinations of surgery, chemotherapy, radiation therapy, stents, endoscopic laser therapy, and photodynamic therapy (PDT) (Enzinger and Mayer, 2003). Stenting is the most common form of palliation for patients with an advanced unresectable esophageal adenocarcinoma.

Endoscopic surveillance of BE

Histologic grade of dysplasia has historically been the standard for assessing disease progression in BE. Biopsy samples are classified (in order of increasing abnormality) negative for dysplasia, indefinite, low-grade dysplasia, high-grade dysplasia (HGD) or cancer. However, each category has biologic heterogeneity; some progress, some regress, and others remain stable, and interpretation of dysplasia is subject to substantial inter- and intraobserver variation (Montgomery et al., 2001). HGD is typically considered to represent such high risk for coexisting or future EA that esophagectomy is frequently recommended (Collard, 2002). However, HGD has substantial biological heterogeneity, and many cases remain stable or even regress. For example, the 5-year cumulative incidences of cancer in patients with HGD range from 9% to 59% in different studies, and two studies have reported regression of HGD in 38%–47% of cases (for review, see Reid et al., 2003). Further, most BE patients have diagnoses less than HGD, and rates of progression for negative, indefinite, and low-grade are low and show no significant differences from each other (Reid et al., 2000). Thus, there is a need for identification of objective, molecular markers for EA risk prediction.

Molecular pathways

BE is a unique model for the study of human neoplastic progression in vivo. Many premalignant tissues are removed when they are detected (e.g., colonic polyps), whereas others cannot be directly visualized (e.g., pancreatic cancer) or densely biopsied because of clinical complications (e.g., bleeding or pneumothorax in the lung). In contrast, the standard of care in BE calls for biopsies to be obtained according to defined protocols at multiple time points from the same patient, allowing generation of spatial maps and longitudinal evaluation of the genetic alterations that arise during clonal evolution. These genetic data can also be linked to epidemiological measures of risk and protective factors in novel multidisciplinary research approaches to investigate neoplastic progression in humans in vivo. The mechanistic knowledge gained from these studies provides scientific advances for early detection and prevention of EA.

However, characterization of molecular alterations in BE and EA has lagged in comparison to other GI cancers for several reasons, including the lower incidence of EA, which makes it difficult for any center to accumulate large numbers of cases, as well as a dearth of easily manipulable cell culture and animal models. Many components of major cell signaling and cell cycle control pathways have been implicated in small studies, including c-erbB2, EGFR, SRC, K-ras, cyclin D1, p16, p27, APC, p53, and telomerase. However, most of these markers are currently in preliminary stages of biomarker development, and relatively little is known concerning their prevalence, stage of development, and mechanistic roles in progression to EA. Although expression arrays hold the promise of identifying genes associated with the development of EA, the statistical complexities of arrays mean that the small number of EAs used in most studies will limit the strength of the conclusions that can be drawn (Ransohoff, 2004).

Neoplastic progression in BE and EA is characterized by chromosomal instability, the most likely cause of which is duodenogastroesophageal reflux. Acid and bile in reflux cause direct tissue damage and inflammation, resulting in the production of reactive oxygen species and nitric oxide. Oxidative damage is a potent mutagen and has been implicated in loss of heterozygosity (LOH) and other chromosomal rearrangements. When taken together, allelotyping, fluorescent in situ hybridization, and comparative genomic hybridization (CGH) analyses demonstrate that a high degree of genetic instability characterizes progression to EA. Nearly every chromosome arm has been identified as undergoing loss or gain during disease progression, due as much to genetic heterogeneity as to differences in laboratory methods and patient populations. This extensive genetic instability can lead to confusion concerning molecular pathways, because neutral mutations not critical for tumor development may “hitchhike” on clonal expansions of selected lesions (Maley et al., 2004). However, several molecular abnormalities have been detected repeatedly in multiple studies and have known biological relevance to the development of EA.

In EA samples, LOH on chromosomes 5q, 9p, 13q, 17p, and 18q has been found frequently and has been attributed to losses of the APC, p16 (CDKN2a), Rb, p53, and DPC4/Bcl2/DCC genes, respectively (see Jenkins et al., 2002; McManus et al., 2004 for reviews). Gain of chromosomes 7 and 11 and loss of 4, 18, and Y has also been reported in CGH studies. Expression array studies have found alterations in a number of genes, including those involved in G2/M processes, such as chromosome condensation and segregation, providing a mechanistic basis for the observed chromosomal instability.

Genes involved in cell signaling and growth pathways have also been reported to be disrupted during progression to EA. Overexpression of products from the c-*erbB2* gene has been found in a minority of EA and is thought to be a later event in progression. EGFR, and to a lesser extent its binding partner TGF-α, are overexpressed in EA. Alterations in oncogenes do not seem to play a prominent role in disease progression in BE, although small studies have reported SRC tyrosine kinase activation, K-*ras* mutations, and mutations in components of the MAPK kinase pathway in EA. Alterations in components of the WNT signaling pathway have been found in BE, including APC (5q LOH, promoter hypermethylation), E cadherin (LOH, reduced expression), and β catenin (mislocalization), although not as commonly as in colorectal cancer.

Methylated APC DNA has been found in the plasma of patients with EA and may be useful as a biomarker of a poor prognosis.

Genes involved in cell cycle control pathways appear to be frequent targets during progression to EA. The retinoblastoma pathway is targeted through LOH (Rb at 13q and p16 at 9p), amplification (cyclin D1), and mutation and promoter hypermethylation (p16). p16 alterations (LOH, mutation, or promoter hypermethylation) are the earliest known genetic/epigenetic lesions in BE, occurring in 90% of BE segments at even the earliest stages of BE (Eads et al., 2000; Wong et al., 2001). Two other CDK inhibitors, p21 and p27, have been reported to be over- and underexpressed, respectively. p53 alterations (LOH, mutation) are found in nearly all EA, and *mdm2* overexpression may also contribute to lack of p53 function.

Apoptosis has been observed to be downregulated during progression to EA; *Bcl2* may play a role early during progression. Telomerase has been found to be activated in nearly all EA, but its relationship to other genetic events is not known. Given the prominent role inflammation plays in the genesis of BE, it is not surprising that cyclo-oxygenase 2 (COX-2) levels are elevated in BE and EA, providing an attractive target for chemoprevention. Promoter hypermethylation has been observed at specific genes (e.g., p16, APC), and while there does not appear to be a global hypermethylation phenotype in BE, extensive hypermethylation may be an indication of poor prognosis for cancer patients.

Validating biomarkers for early detection

The National Cancer Institute's Early Detection Research Network has recently defined five phases of biomarker development (Sullivan Pepe et al., 2001). Although there are more than 200 candidate biomarkers in BE, including those identified in array studies, more than 95% are in Phase 1 (Discovery). A handful of markers, including 17p LOH, cyclin D1 immunostaining, tetraploidy, and aneuploidy, have undergone phase 3 or phase 4 validation, suggesting their utility as intermediate markers of progression. These markers also represent disruption of some of the major molecular pathways altered in human cancer and provide a framework for the study of other genetic and epigenetic alterations.

p53

p53 alterations (mutation, LOH) occur later in disease progression than p16 alterations and may be more directly linked with increased risk of progressing to EA. p53 mutations have been evaluated by DNA sequencing and by p53 immunohistochemistry as a surrogate; DNA sequencing is preferable because of high false-positive and false-negative rates for p53 immunohistochemistry. In one well-designed phase 3 validation trial, cyclin D1 overexpression was associated with progression to EA, whereas p53 immunostaining was not (Bani-Hani et al., 2000). In prospective studies, patients with 17p (p53) LOH detected by genotyping have a substantially increased risk for progressing to EA compared to those patients with two 17p alleles (relative risk = 16 [95% CI, 6.2-39, $p < .001$], reviewed in Reid et al., 2003). p53 alterations are also mechanistically associated with the development of tetraploidy and aneuploidy.

Tetraploidy and aneuploidy

Inactivation of p53 in BE predisposes to the development of a genetically unstable tetraploid cell population that predicts progression to aneuploidy and EA. Two phase 4 studies from different institutions have reported that DNA content flow cytometry can help resolve the biological heterogeneity in Barrett's patients without high-grade dysplasia (where standard diagnostics may miss high-risk patients) by identifying a low-risk patient population with a 0% chance of cancer and a high-risk population with approximately a 25% incidence of cancer within five years (Reid et al., 2000; Teodori et al., 1998).

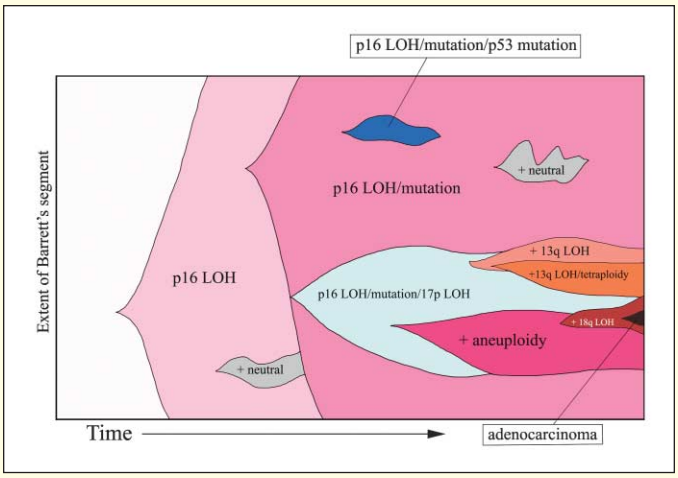

Figure 2. Clonal evolution of esophageal adenocarcinoma in Barrett's esophagus

An example of how clonal evolution in Barrett's esophagus generates genetic heterogeneity during progression to EA. The x axis is time and the y axis represents the Barrett's segment. Clones with p16 alterations are common early events and frequently spread to encompass large regions of the Barrett's segment. Within these expansions, additional p16 alterations, alterations involving p53 (17p LOH or mutation), and flow abnormalities can occur. Neutral mutations provide no selective advantage and expand or contract stochastically, although some neutral mutations may expand as "hitchhikers" on an expanse mediated by a p16 lesion. Alterations on other chromosomes are likely to be selected during neoplastic progression; e.g., chromosome 18 LOH, which typically develops before EA, and LOH on chromosomes 5q and 13q, which can occur before or after the development of cancer. Note that some clones may disappear over time as a result of clonal competition from other selective sweeps that drive them to extinction. Although this diagram indicates frequently observed events in the development of EA (e.g., p16 alterations occurring early, cancer arising in clones with flow abnormalities), not all events occur or occur in this order in all cancers.

Based upon the principal genetic alterations found in BE and EA (e.g., see Jenkins et al., 2002), a general picture is emerging of the evolution of EA in patients with BE (Figure 2). Very early, most patients develop clones with p16 alterations that frequently expand throughout the Barrett's segment and which may influence the length of the segment (Wong et al., 2001). Progeny clones with p53 lesions typically evolve from p16-deficient progenitors and predispose to evolution of tetraploidy, aneuploidy, and EA. Alterations in other cell cycle control genes, oncogenes, telomerase, and others certainly contribute to this progression, but are not sufficiently well-characterized to speculate how they contribute to EA. The genetic alterations are frequently independent of histologic grade, although there is likely some nonlinear correlation between molecular alterations and histologic abnormalities. Clonal ordering, a method for comparing the order in which two clonal genetic abnormalities develop during neoplastic progression, is amenable to analysis of small sample sets and could be widely used to improve our understanding of the roles of other genes in progression to EA (reviewed in Reid et al., 2003). Future studies may elucidate the molecular bases for the histologic changes observed in BE.

Recent advances in diagnosis, treatment, and prevention

Screening

Most EAs are detected in patients who are not known to have BE prior to the cancer diagnosis. Therefore, present American College of Gastroenterology guidelines recommend screening patients with chronic reflux for BE (Sampliner, 2002). These guidelines have been challenged because the number of individuals with reflux in the population is large relative to the absolute risk of EA, and it has been suggested that the risk of complications during screening endoscopy, a generally safe procedure, may outweigh the benefit (Shaheen and Ransohoff, 2002). Preliminary feasibility studies with a video capsule that can be swallowed show promise as a screening tool, although large studies are presently lacking. There are presently no validated serum tests for BE or EA.

Surveillance and treatment

While the current standard of care for patients with BE is periodic endoscopic surveillance with biopsy for early detection of cancer, the role of surveillance in reducing mortality associated with EA remains controversial. Multiple reports in the literature indicate that intensive surveillance programs that use jumbo, four-quadrant biopsies every cm, targeting suspicious visible lesions with interpretation by an experienced GI pathologist, can detect early-stage EA with improved survival (Corley et al., 2002; Schnell et al., 2001). However, other studies have reported that no curable cancers were detected despite years of surveillance (Conio et al., 2003). This is likely because many elements of surveillance are operator-dependent, including the quality of endoscopic biopsy sampling, pathologic interpretation of dysplasia, and institutional experience with high-risk patients. It is possible that the level of care required for the detection of early cancer using present surveillance techniques can only be achieved consistently at specialized, high-volume institutions.

A range of research advances have been directed toward improving surveillance, including development of molecular diagnostics (described above) and improved endoscopic detection. A number of different endoscopic modalities have also been developed as adjuncts to or replacements for biopsy for detection of EA. Rather than relying exclusively on visual inspection and systematic biopsy protocols of the Barrett's segment, these new methods, including optical coherence tomography, narrow band imaging, and Raman spectroscopy, may allow identification of high-risk lesions (Bouma et al., 2000; Kendall et al., 2003). Techniques like Raman spectroscopy and narrow band imaging also have the advantage of surveying the entire Barrett's segment, rather than depending upon chance that an important lesion will be sampled by biopsy, and may provide additional information that can guide endoscopic biopsy for identifying EA. An eventual goal of these technologies is to develop an optical biopsy to detect spectral or other signatures indicative of EA or the risk of developing it that would not be visible during routine endoscopy.

Endoscopic therapies are also being investigated as alternatives to esophagectomy for HGD and EA. Endoscopic mucosal resection removes sections of esophageal epithelium and submucosa to the muscularis propria (Pacifico and Wang, 2002), potentially resecting early cancers that can be evaluated microscopically to assure the margins are free of cancer. However, residual BE remains at risk for EA. Ablation of Barrett's epithelium combined with effective acid suppression therapy frequently results in the regrowth of apparently normal squamous epithelium. Thermal photocoagulation techniques, such as multipolar electrocoagulation and argon plasma coagulation, which destroy the intestinal epithelium, mucosal resection, and PDT, in which a photosensitizing agent that accumulates in the epithelium is activated by an endoscopic light source, are currently being evaluated as alternatives to esophagectomy. These treatments are promising, but not without drawbacks. Complications such as systemic photosensitivity or esophageal strictures can occur with PDT, and residual BE remains in the majority of cases (Selvasekar et al., 2001), sometimes hidden underneath the neosquamous epithelium. Published data from clinical trials with five-year follow-up are not yet available to determine the effectiveness of ablative treatments in preventing progression to EA. However, available data indicate it should not be considered curative and surveillance should be continued indefinitely because residual BE can progress to EA, and there have been reports of malignancies developing in BE concealed beneath the neosquamous epithelium. There is also evidence that some p53 mutant or aneuploid clones have relative resistance to PDT (Krishnadath et al., 2001).

Given that most patients with EA present with advanced disease, therapies in addition to surgery have been investigated to improve survival (Burak, 2003; Enzinger and Mayer, 2003). Phase III trials of preoperative chemotherapy (5-FU and cisplatin) failed to show improved survival over surgery alone. Since 1996, regimens combining chemo- and radiation therapy have been examined for efficacy in treating EA. The results of these studies have been equivocal; while approximately 1/4 of patients treated have a complete pathologic response to neoadjuvant chemo/radiotherapy, there have been conflicting reports whether overall survival is significantly improved or if any improvement is offset by increased surgical morbidity or mortality (reviewed in Burak, 2003).

Prevention

Given the typical late onset of EA, treatments that delay progression of BE to EA are attractive options for reducing the morbidity and mortality associated with EA and esophagectomy. Nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, have been shown to be associated with a decreased risk of EA in epidemiology studies and animal models, and they have been associated with reduced 17p LOH in cross-sectional studies of patients with BE (Corley et al., 2003; Vaughan et al., 2002). Clinical trials are currently underway to determine if COX-2 inhibitors such as celecoxib are effective in reducing dysplasia in BE. A recent cross-sectional study of patients with BE reported that those with higher serum selenium levels had lower 17p LOH, tetraploidy, and aneuploidy (Rudolph et al., 2003). A diet high in fruits and vegetables has also been consistently associated with lower risk of EA in epidemiology studies (Brown and Devesa, 2002).

Future challenges

Esophageal cancers are uncommon in the United States, and most centers only see a small number of cases, resulting in small studies that are underpowered statistically to address the complexity of molecular evolution within the Barrett's segment and to conduct definitive clinical trials, including early detection and prevention research. Creation of interdisciplinary, multicenter networks to address these issues is a challenge that was recognized and reviewed in the NCI Stomach/Esophageal Progress Review Group (http://prg.nci.nih.gov/stomach/finalreport.html).

Our limited understanding of the biochemical pathways altered in BE is due in part to the relative paucity of cases seen in single centers combined with difficulties in growing Barrett's epithelium in culture and recapitulating the environment of the acid exposed esophagus in animal models. While animal models exist (rat, dog, rabbit) for the development of BE (Koak and Winslet, 2002), they generally require nonphysiologic conditions to initiate reflux (e.g., surgical esophagoduodenal anastamosis) and are not in models amenable to genetic manipulation, such as the mouse. Development of physiological tissue culture and animal models are of great importance for more complete understanding of the genetic pathways involved in the development of BE and EA.

BE and EA are challenges at both the population and patient level. The rapid increase in the incidence of EA over the past 30 years in the US and other Western countries, combined with its association with GERD and obesity, are concerning. Given the number of people in the US alone who have frequent GERD and the percentage of obese children and adults, we may just be seeing the tip of the iceberg for BE and EA that will require population-based interventions for effective control. Management of the individual patient with BE is another key question, given limited treatment dollars, because most patients with BE will not develop EA, and the money that goes into their surveillance does nothing to improve their outcome. Yet the patients who do progress to EA face a grim future unless the cancer is detected before it has begun to spread, and less than 10% of patients would be expected to survive for 5 years after developing a metastatic EA. The main challenge is to stratify patients so that those at high risk can be enrolled in surveillance programs and those at low risk can be counseled, reassured of their low risk, and monitored safely at much greater intervals.

Acknowledgments

The authors apologize that space restrictions prevent a more complete listing of the studies that have advanced research in the fields of Barrett's esophagus and esophageal adenocarcinoma. Readers are referred to recent reviews and a peer-reviewed website for a more thorough discussion of clinical aspects (Enzinger et al., 2003; Sampliner, 2002; and http://www.barrettsinfo.com), epidemiology (Brown and Devesa, 2002), and markers of disease progression (Jenkins et al., 2002; McManus et al., 2004; Reid et al., 2003). The authors have been supported by grants from the National Institutes of Health, including K07CA089147 (T.G.P.) and P01CA91955 (B.J.R.).

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