

## Biomarkers in Exploring the Frontiers of Diagnosis, Prognosis, and Therapy of Barrett's Esophagus

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### Abstract

Barrett's esophagus (BE) is the principal risk factor for esophageal adenocarcinoma. BE patients currently undergo periodic endoscopic surveillance with tissue sampling and histopathologic assessment for dysplasia. They frequently are prescribed proton pump inhibitors to pharmacologically suppress gastric acid that is the cause of BE. These standard endoscopic and pharmacologic approaches for managing BE are crude at best. Identification of novel tissue biomarkers within BE may allow for more accurate endoscopic risk stratification and provide potential targets for chemoprevention. *Cancer Prev Res*; 4(6): 783–6. ©2011 AACR.

Barrett's esophagus (BE) is defined as the replacement of native esophageal squamous mucosa with specialized intestinal metaplasia containing columnar epithelium with prominent goblet cells. This metaplasia occurs in response to chronic esophageal acid exposure, typically in individuals with long-standing gastroesophageal reflux disease (GERD).

BE is an asymptomatic condition; however, its clinical significance is directly related to cancer risk because BE is the strongest known risk factor for esophageal adenocarcinoma (EAC; ref. 1). EAC is the 4th most common gastrointestinal malignancy in the United States (2), and its incidence is rising at a rapid and alarming rate. Patients with BE are typically enrolled in endoscopic surveillance programs for early cancer detection. The risk of EAC is predicated in the presence or absence of dysplasia in BE tissue within endoscopically obtained biopsies. Under current histopathologic grading criteria, BE may be characterized as nondysplastic intestinal metaplasia, metaplasia containing low-grade dysplasia (LGD), or metaplasia containing high-grade dysplasia (HGD). Progression along this continuum, prior to the development of intramucosal and/or invasive adenocarcinoma, may not necessarily be unidirectional or stochastic.

The risk of EAC in patients with BE is approximately 0.5% to 1% per year (3), but this risk is increased when the BE harbors foci of dysplasia. Meta-analysis data suggest a 6% to 7% annual cancer risk in patients with BE-HGD (4);

however, a recent randomized trial of endoscopic radiofrequency ablation therapy for BE-HGD found a 19% cancer progression rate at 12 month follow-up in the sham treatment arm (5). Therefore, the presence of HGD is a trigger for intervention, either with endoscopic therapy or surgical esophagectomy.

Proton pump inhibitors (PPIs) are commonly prescribed for patients with BE to provide pharmacologic suppression of gastric acid. Although the ostensible purpose of PPIs is to eliminate gastroesophageal acid exposure and perhaps control symptomatic GERD, this therapy may be broadly defined as chemopreventive. PPI use has been associated with a reduced likelihood of progression of BE to cancer in retrospective cohorts (6, 7). Direct evidence supporting an effect of PPI therapy on regression of established dysplasia is limited, although regression of HGD was seen at 5 years in 39% of a control group treated with PPI therapy alone in a randomized trial of photodynamic therapy for BE-HGD (8).

The aforementioned endoscopic and pharmacologic approaches comprise current clinical staging and practice in the management of BE, but are crude at best. HGD is an imperfect marker for risk stratification or grading of disease severity and is prone to biopsy sampling error (9, 10) and subjective, variable histopathologic interpretation (11). PPIs suppress gastric acid production at the level of the hydrogen potassium adenosine triphosphatase ( $H^+/K^+$ -ATPase) in gastric parietal cells, and have no known direct effect or action on Barrett's epithelium; moreover, elimination of gastric acid may not fully protect the esophagus from the mutagenic potential of esophageal refluxate, given the ever deepening understanding of the molecular effects of bile salts on esophageal cells (12).

The future of BE diagnosis and therapy will (and must) center instead of the identification and exploitation of novel tissue biomarkers. The ideal BE biomarker would (i) serve as a tool for endoscopic diagnosis and risk stratification and (ii) offer a target for pharmacologic

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chemoprevention. In this issue of the journal, Sinicrope and colleagues (13) report an important effort toward the latter biomarker function, with their study of the effects of difluoromethylornithine (DFMO) on mucosal polyamines, gene expression, and histopathology in BE (LGD and HGD). Prior study, some more than 2 decades old (14), showing growth inhibition by DFMO in Barrett's-derived *ex vivo* tissue systems and increased activity of ornithine decarboxylase (a target of DFMO) in BE (14) supported the rationale for the new study.

Sinicrope and colleagues found that DFMO at a daily dose of 0.5 g/m<sup>2</sup> for 6 months significantly suppressed levels of the polyamines putrescine and spermidine in mucosal biopsies at 6 months in 10 patients with BE-LGD; this effect seemed to be maintained for 6 months following discontinuation of therapy. Genes modulated by DFMO included those encoding the cell-cycle regulators cyclin E2 and plexin B1, *RPL11*, which is involved in p53 signaling, and the transcription factor Krüppel-like factor 5.

Of interest, complete regression of LGD occurred at 6 months in 1 patient, and partial regression (from multifocal to focal) of LGD in 2 other patients. In this regard, the authors appropriately acknowledge the potential for confounding by biopsy sampling error, acid suppression, or spontaneous regression. Compounded by these confounding factors, the inclusion of patients with LGD in this study represents another limitation, given the rarity of a secure diagnosis of LGD. In a recent study from an expert center in endoscopic therapy of BE, only 15% of 147 patients referred with a diagnosis of LGD were confirmed to have LGD by expert histopathology review, with the remainder being downstaged to nondysplastic BE (15). Dysplasia of any degree as an endpoint for a BE intervention study of any scale or scope must be recognized as no better than a surrogate endpoint along the continuum to EAC.

The findings of Sinicrope and colleagues are clinically important and should provide the impetus for further study; however, the ultimate hurdle for targeted molecular chemoprevention in BE, no matter how attractive or accessible the target, will be its ultimate application to clinical practice. Nowhere has this hurdle been better illustrated than with COX-2. COX-2 expression is upregulated in BE, with and without dysplasia, and EAC (16–18) and correlates in both with expression of additional genes involved in tumorigenesis, including the antiapoptotic gene *bcl-2* (19) and the gene encoding epidermal growth factor receptor (20). Furthermore, the degree of COX-2 overexpression may have prognostic implications for metastases and overall survival in EAC patients (21–22). COX-2 expression levels in neosquamous epithelium return to baseline in BE-HGD patients following endoscopic treatment with photodynamic therapy (23). As a target of pharmacologic intervention for BE, however, COX-2 has been less than adequate.

The multicenter chemoprevention in BE trial (CBET) randomly assigned 100 patients with BE containing LGD or HGD to receive celecoxib (200 mg twice daily) or placebo for 48 weeks (24). Concomitant PPI therapy

was allowed. This study failed to identify differences in prostaglandin levels or COX-2 mRNA expression in BE tissue between the celecoxib and placebo groups. The study also failed to identify differences in the primary outcome, defined as a change in proportion of biopsies showing dysplasia, between the celecoxib and placebo arms. The CBET did not reach its accrual goal, and whether the null results are due to a lack of power and type II error, or rather, to inadequate dosing and duration of celecoxib, is uncertain. Another randomized trial, the phase II aspirin esomeprazole chemoprevention trial (AspECT; NCT00357682), is testing a nonselective COX inhibitor, is based in the United Kingdom, and has a recruitment goal of 5,500 subjects; interim results of this ongoing trial are expected in 2011.

The experience with COX-2 in BE is also germane to safety aspects one must consider in a potential chemoprevention strategy. The adverse cardiovascular events associated with certain selective COX-2 inhibitors have now been well publicized. Patient preferences for the use of COX-2 for EAC chemoprevention were addressed in a study by Hur and colleagues (25); 100 patients with BE were administered a questionnaire with visual aids identifying options for chemoprevention. The patients were instructed that medicine A (celecoxib) taken once daily (i) would lower their lifetime risk of developing esophageal cancer from 10% to 5% and (ii) would also increase their lifetime risk of myocardial infarction from 50% to 75% and increase their lifetime risk of stroke from 20% to 30%. They were further instructed that medicine B (aspirin) taken once daily would decrease their lifetime risk of esophageal cancer from 10% to 5% and decrease their lifetime risk of myocardial infarction from 50% to 35%. Potential adverse gastrointestinal side effects of celecoxib and aspirin were also included in the scenario. Not surprisingly, 76% of the patients in this base-case analysis expressed a willingness to take aspirin, whereas only 15% were willing to take celecoxib. The majority of subjects unwilling to take celecoxib identified increased risk of myocardial infarction as the primary factor for declining this chemopreventive option. Subsequent sensitivity analyses revised the celecoxib scenario to a perfect EAC chemoprevention strategy (100% elimination of EAC risk) and retention of an increased risk for myocardial infarction; still, only 43% of subjects were willing to take celecoxib (25).

This study by Hur and colleagues underscores the importance of holding pharmacologic chemopreventive measures to a high standard of both efficacy and safety, not least in the eyes of patients; the success of a chemoprevention strategy hinges in large part upon patients' threshold for tolerating exposure to potential pharmacologic risks. With regard to this critical consideration, Sinicrope and colleagues examined the potential for DFMO-associated ototoxicity. Patients underwent audiometric evaluation at baseline and again following 6 months of DFMO exposure. Audiometric changes were documented in 3 patients, and changes in distortion product otoacoustic emission, described as a sensitive indicator of potential ototoxicity,

were documented in all patients (13). Certainly, the long-term clinical consequences of this or a greater degree or magnitude of exposure to DFMO will require further attention as this clinical pilot work is carried forward. Hopeful safety signals for this future study come from 2 long-term placebo-controlled clinical trials involving DFMO, 1 in skin cancer prevention that found reasonable safety and mild ototoxicity with DFMO at 500 mg/m<sup>2</sup>/day (same as dose of Sinicrope and colleagues) for 4 to 5 years (26), the other in colorectal adenoma prevention that found a real but subclinical increase in ototoxicity with a lower dose (500 mg/day) of DFMO (combined with sulindac) given for 3 years (versus placebo; ref. 27).

Even if a rigorously vetted, thoroughly efficacious, and safe pharmacologic agent were available for chemoprevention of EAC, the following major hurdle to widespread clinical application remains accurate identification of individuals at risk. The majority of individuals with symptomatic GERD will never develop BE; conversely, some individuals with pathologic GERD experience no episodes of symptomatic heartburn and are incidentally found to have BE during upper endoscopy for other indications. Clinical practice has shown that BE is prevalent in individuals both with and without symptomatic GERD. A study of outpatients referred for colonoscopy who consented to a screening upper endoscopy found that the prevalence of BE was 8.3% in patients with and 5.6% in those without a history of heartburn (28). On the basis of such data, symptom-targeted screening or chemopreventive programs would clearly fail to identify a sizable asymptomatic population at risk. Meanwhile, formal practice guidelines from the major North American gastroenterology societies have become increasingly vague with respect to recommendations for screening endoscopy. The American Gastroenterological Association practice guidelines, for example, cite insufficient evidence to recommend either for or against endoscopic screening for BE in individuals 50 years or older with a history of more than 5 to 10 years of heartburn (29).

If the accurate and comprehensive identification of individuals with BE is not feasible, is there an alternative to a proactive chemopreventive strategy? Although the

reasons for the increasing incidence of BE and EAC are not fully understood and although increasing clinical recognition of the former may be in part due to detection bias given the widespread growth of open-access endoscopy, compelling data point to an inverse association between BE and infection with *Helicobacter (H.) pylori*. In a recent epidemiologic study derived from a U.S. endoscopy database with nearly 250,000 individual patients, Sonnenberg and colleagues showed a significantly decreased OR of BE (OR = 0.58; 95% CI, 0.49–0.69) in individuals with biopsy-confirmed *H. pylori* infection, chronic gastritis, or gastric intestinal metaplasia (30). An editorial accompanying this study provocatively questioned whether *H. pylori* may be protective with respect to the esophagus, whether the absence of *H. pylori* predisposes to development of GERD, whether the decline in the prevalence of *H. pylori* and increased prevalence of GERD are "linked," and whether indiscriminate pharmacologic eradication of *H. pylori* infection is appropriate (31). By the rationale that *H. pylori* could be protective, the ideal chemopreventive strategy for BE might consist of less rather than more.

Although the medical and scientific communities continue to investigate the pathogenesis and bimolecular basis of BE and grasp at the tantalizing prospect of chemoprevention, the fact remains that BE is an increasingly prevalent condition. Individuals with BE, many of whom will never go on to develop EAC, are subjected to lifelong invasive endoscopic surveillance in an effort at early detection of malignancy. When EAC is undiagnosed until a symptomatic stage, it carries an unfavorable and often incurable prognosis. Answers to the questions posed before, and improved efforts to abrogate progression to malignancy, will be eagerly awaited by patients with BE and their health care providers alike.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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