New Strategies in Barrett’s Esophagus: Integrating Clonal Evolutionary Theory with Clinical Management

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Abstract

Barrett’s esophagus is a condition in which the normal stratified squamous epithelium of the distal esophagus is replaced by intestinal metaplasia. For more than three decades, the prevailing clinical paradigm has been that Barrett’s esophagus is a complication of symptomatic reflux disease and predisposes to esophageal adenocarcinoma. However, no clinical strategy for cancer prevention or early detection based on this paradigm has been proven to reduce esophageal adenocarcinoma mortality in a randomized clinical trial in part because only about 5% to 10% of individuals with Barrett’s esophagus develop esophageal adenocarcinoma. Recent research indicates that Barrett’s metaplasia is an adaptation for mucosal defense in response to chronic reflux in most individuals. The risk of progressing to esophageal adenocarcinoma is determined by development of genomic instability and dynamic clonal evolution in the distal esophagus modulated by host and environmental risk and protective factors, including inherited genotype. The challenge for investigators of Barrett’s esophagus lies in integrating knowledge about genomic instability and clonal evolution into clinical management to increase the lifespan and quality of life of individuals with this condition. Clin Cancer Res; 17(11); 3512–9. ©2011 AACR.

Background

In individuals with Barrett’s esophagus, the distal portion of the normal esophageal stratified squamous epithelium is replaced by specialized intestinal metaplasia (1). The paradigm that Barrett’s esophagus arises as a complication of chronic symptomatic gastroesophageal reflux disease and predisposes to esophageal adenocarcinoma has dominated clinical thought for more than three decades (2, 3). Practice guidelines have endorsed endoscopic screening of individuals with symptomatic reflux to detect Barrett’s (4), endoscopic biopsy surveillance of Barrett’s for early detection of esophageal adenocarcinoma (1, 4), and intervention typically reserved for individuals with high-grade dysplasia or cancer (1, 4). However, the rate of progression from Barrett’s esophagus to esophageal adenocarcinoma is low, and consequently 90% to 95% of individuals diagnosed with Barrett’s esophagus follow a benign course, living out their lives without developing or dying of esophageal adenocarcinoma (5–9). Current strategies to decrease the cancer risk in Barrett’s esophagus do not take into account these low rates of progression to (and death from) esophageal adenocarcinoma. These strategies are further compromised by the use of morphologic assessment of dysplasia for risk assessment in Barrett’s esophagus. Although dysplasia is frequently used as a surrogate endpoint in Barrett’s esophagus research, neither high-grade dysplasia nor any other grade of dysplasia fulfills criteria for valid surrogates. This is the case because dysplasia does not accurately represent the true endpoint (esophageal adenocarcinoma); because dysplasia classification is subjective, not reproducible; and because it does not provide robust discrimination between individuals who will regress or remain stable for prolonged periods and those who will develop life-threatening disease (10–12).

Although some data support endoscopic biopsy surveillance for early detection (1, 12, 13), aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) for chemoprevention (14, 15), and medical surgical interventions including ablation for cancer control in individuals with Barrett’s esophagus (16–19), to date no randomized control trials have convincingly shown a reduction in the incidence and mortality of esophageal adenocarcinoma (1, 12). In summary, no management strategy for early detection or prevention based on the prevailing clinical paradigm has yet been proven to reduce the mortality of esophageal adenocarcinoma or all-cause mortality.

On the Horizon

Multilevel evolution in Barrett’s esophagus

In this article, we explore an overarching evolutionary theory of the development of Barrett’s esophagus and its
progression to esophageal adenocarcinoma. This theory incorporates inherited changes in the constitutive genome and clonal somatic genomic instability in Barrett's epithelium that predispose to esophageal adenocarcinoma. In other words, there has been natural selection at the level of individuals in our ancestors, and there is ongoing selection at the level of cells in the Barrett's epithelium. As part of the overarching evolutionary theory, we examine a relatively new theory that specialized intestinal metaplasia is a successful adaptation to gastroesophageal reflux that remains stable for the lifetime of most individuals. This theory suggests that the propensity to develop specialized metaplasia in response to gastroesophageal reflux is the product of natural selection at the level of individuals and natural selection at the level of cells in a reflux environment.

**Defining the constitutive genome from which somatic clones evolve.** At the level of the individual, there is substantial evidence for an inherited component to Barrett's esophagus and esophageal adenocarcinoma based on case reports, twin studies, familial clusters and clinical series (20–22). For example, families have been described that show strong predispositions to esophageal adenocarcinoma, Barrett's esophagus, and reflux disease (21, 23). Studies of reflux disease in twins suggest heritability of 30% to 40%, and twins have been reported to develop Barrett's esophagus, suggesting a role for genetic susceptibility in these conditions (24–26). Larger studies support a role for genetic susceptibility for Barrett's esophagus and esophageal adenocarcinoma (27). In one study, 7.3% of individuals presenting with Barrett's esophagus or esophageal adenocarcinoma were reported to have a familial component (22). Practice guidelines for physicians who treat individuals with Barrett's esophagus and esophageal adenocarcinoma now recommend obtaining a family history (28). Advances in whole-genome sequencing of family members (29) will greatly accelerate discovery of inherited genetic alterations that predispose to Barrett's esophagus, esophageal adenocarcinoma, or both. Once identified, these individuals could be enrolled in trials to prevent esophageal adenocarcinoma or detect it when it is early and curable.

In 1976, Nowell described another level of evolution when he hypothesized that "acquired genetic lability permits stepwise selection of variant sublines and underlies tumor progression" (30). At this level, clonal evolutionary theory focuses on the genetics of the evolving clonal populations in the distal esophagus, their interactions with each other, and their relationship to the native esophageal squamous epithelium. There is substantial evidence that evolution of esophageal adenocarcinoma is associated with potentially modifiable host and environmental risk (e.g., obesity and cigarette smoke) and protective (e.g., aspirin and other NSAIDs) factors in the population (12).

**Genomic instability, clonal evolution, and clonal evolutionary parameters in Barrett's esophagus.** The genomes of esophageal adenocarcinomas contain complex alterations that disrupt regulatory pathways and are associated with profound changes in the transcriptome and proteome (12, 31–34). Several lines of evidence indicate that these abnormalities arise by a process similar to that postulated by Nowell, including genomic instability, generation of genetic variants, natural selection, and dynamic evolution of clones (12). Most esophageal adenocarcinomas (on the order of 90% to 95%) arise in association with chromosome instability that leads to gains, losses, or loss of heterozygosity (LOH) of large regions of chromosomes (31, 35, 36). Studies suggest that evaluation of the observable patterns generated by clonal evolution, including chromosomal alterations and instability, temporal order of mutation events, clonal genetic diversity, and clonal expansions, may facilitate risk assessment in Barrett's esophagus by means of novel approaches that can be translated to the clinic and potentially to other cancers.

Spatial data from Barrett's esophagus and esophageal adenocarcinoma from the same patient at the same time have been used to develop models of clonal evolution, leading to the hypothesis that 9p LOH (and CDKN2A mutation and methylation) are early events, occurring before 17p LOH (and TP53 mutations), which predispose to development of DNA content abnormalities (tetraploidy, aneuploidy) during evolution to esophageal adenocarcinoma (37).

9p and 17p LOH, CDKN2A mutations and methylation, and TP53 mutations have been found to be highly associated with clonal expansions, suggesting that these alterations provide a selective advantage to a Barrett's clone (38). However, 9q LOH and 17q LOH, as well as microsatellite shifts, behave as neutral genetic abnormalities. Although neutral chromosome changes are occasionally detected in clonal expansions, these changes typically represent hitchhikers ("passengers") on known selected genomic abnormalities ["drivers" (ref. 38)]. Increasing sizes of clones with 17p (TP53) LOH, tetraploidy, and aneuploidy are associated with increasing risk of progression to esophageal adenocarcinoma, but sizes of clones with CDKN2A abnormalities are not associated with such risk after controlling for 17p LOH (39). This result supports the hypothesis that expansion of a genetically unstable clone increases the risk of progression to esophageal adenocarcinoma. One prediction based on this hypothesis is that the genetically unstable clone will produce viable variants during an expansion that increases the probability of evolving to cancer. Maley and colleagues (40) evaluated this hypothesis in another study and reported that clonal diversity, as assessed by number of clones, Shannon index, and mean pairwise divergence, was associated with an increased rate of progression to esophageal adenocarcinoma even when 17p LOH and DNA content abnormalities were controlled for in the model. In a follow-up study, Merlo and colleagues (41) evaluated a wide range of diversity metrics from the ecology literature as well as defining clones based on different sets of loci and lesions. They found that every diversity measure and every method for defining a clone produced biomarkers that were highly statistically significant (P < 0.001) predictors of progression to esophageal adenocarcinoma in this cohort. This suggests that diversity measures are robust biomarkers of progression in Barrett's esophagus. Clonal genetic diversity has also been...
reported at the crypt level in Barrett’s esophagus (42), and these preexisting genetic variants could be a source of development of resistance to interventions to prevent cancer. In summary, several elements of Nowell’s theory, including manifestations of genomic (chromosomal) instability, expansion of genetically unstable clones, and generation of viable clonal variants, contribute to clonal evolution from Barrett’s esophagus to esophageal adenocarcinoma.

Specialized intestinal metaplasia and mucosal defense

As important as the genomic results are in identifying individuals at increased risk for neoplastic clonal evolution to esophageal adenocarcinoma, they provide little insight into the reasons why 90% to 95% of individuals will not progress to esophageal adenocarcinoma during their lifetime. Barrett’s esophagus arises in an environment of chronic injury associated with acid and bile reflux into the esophagus (12). Orlando and colleagues proposed a novel theory positing that specialized intestinal metaplasia of the distal esophagus is a successful adaptation for mucosal defense that persists for the lifetime of most individuals (ref. 43; Fig. 1). In a combined expression and proteomic study, Ostrowski and colleagues (44) concluded that "Barrett’s metaplasia may be regarded as a specific microevolution allowing for accumulation of mucosal morphological and physiological changes that better protect against reflux injury." These results suggest that specialized intestinal metaplasia provides a barrier against reflux injury that confers a selective advantage over the native esophageal epithelium, leading to expansion of intestinal metaplasia in the distal esophagus.

Chromosomal instability is associated with disruption of mucosal defense. Two articles published in 1989 evaluated mucus secretion in Barrett’s esophagus by transmission electron microscopy. The first study revealed that specialized intestinal metaplasia typically has active intracellular mechanisms for synthesis and transport of mucus, including glycogen aggregates, rough endoplasmic reticulum, Golgi apparatus, and mucus secretory granules (45).
Barrett’s esophagus thus has a spectrum of morphologic features thought to participate in mucosal defense that overlaps with normal gastric and small intestinal mucosa (45). The second article reported that biopsies of individuals with Barrett’s esophagus who had flow cytometric DNA content abnormalities (tetraploidy or aneuploidy), including some who progressed to esophageal adenocarcinoma, had reduced mucous content as well as striking ultrastructural abnormalities that included distended rough endoplasmic reticulum, increased cytoplasmic glycogen aggregates, small or dysmorphic Golgi apparatus, atypical mucous granules, and a “simplified cytoplasmin” with fewer organelles and mucous granules (46). Currently available data are not sufficient to determine whether genomic instability develops as a consequence of loss of the barrier function with genotoxic injury to stem cells at the base of the crypts, whether chromosome instability leads to disruption of the barrier function of Barrett’s metaplasia, whether these processes are independent of each other, or whether both interact in a vicious cycle that leads to selfish cell proliferation and evolution to esophageal adenocarcinoma.

If the goal of research is to reduce the mortality of esophageal adenocarcinoma and improve the quality of life of individuals with Barrett’s esophagus who will not progress to cancer, then greater accuracy in risk assessment will be required to distinguish between benign early adaptations that will not shorten an individual’s lifespan and clonal evolution of life-threatening early disease. Reid and colleagues (12) proposed a phased approach with a series of risk models involving four strong population-attributable risk factors (obesity, tobacco, reflux, and diet low in fruits and vegetables) for the general population; history, physical examination, and blood tests for primary care; and blood-and-tissue-based biomarkers for secondary care. Although reflux disease and Barrett’s esophagus may inform several of the risk models, this approach differs from the previous paradigm in that it does not depend on the symptomatic reflux → Barrett’s esophagus → esophageal adenocarcinoma paradigm that currently guides clinical management. Here, we focus on the transition from Barrett’s esophagus to esophageal adenocarcinoma as a process of dynamic clonal evolution modulated by both inherited genotype and environmental risk and protective factors.

Converting measures of chromosome instability, chromosomal mutation rate, and clonal diversity to risk-assessment tools. A 10-year prospective cohort study (47) reported that a panel of 9p LOH, 17p LOH, and DNA content abnormalities (tetraploidy and/or aneuploidy) was a strong predictor of progression from Barrett’s esophagus to esophageal adenocarcinoma [relative risk = 38.7; 95% confidence interval (CI), 10.8–138.5; \( P < 0.001 \)]. Individuals with all three manifestations of chromosome instability at baseline had a 79.1% 5-year cumulative incidence of cancer compared with no cumulative incidence (0%) of cancer to nearly 8 years in individuals who had none of the markers.

Rapidly advancing technologies create opportunities for evaluating somatic genomic evolution on a single platform (31), making it more suitable for the clinical laboratory. Single-nucleotide polymorphism (SNP) arrays provide flexible platforms for a variety of approaches to investigate cancer, including genome-wide association studies, customized platforms to assess specific regions of the genome, and a uniform platform for genome-wide assessment of somatic LOH, copy change, and aneuploidy (31, 35, 36).

Identifying evolutionary mechanisms by which candidate chemoprevention agents act and anticipating evolution of resistance for clinical trials. Importantly, current use of aspirin and other NSAIDs in the 10-year prospective cohort study described above was associated with marked risk reduction in patients with two or more chromosome instability biomarkers at baseline. NSAID nonusers had a 79% 10-year cumulative incidence of esophageal adenocarcinoma, compared with 30% for current NSAID users \( (P < 0.001; \text{ref. 47}) \). In a separate study of the same cohort, current use of aspirin and other NSAIDs was associated with decreased progression to DNA content abnormalities (e.g., tetraploidy and aneuploidy) as well as esophageal adenocarcinoma (48). These results support development of clinical trials using aspirin or other NSAIDs. Further studies in observational cohorts could inform these trials by elucidating the evolutionary mechanisms of the NSAID protective association and identifying genomic abnormalities associated with NSAID resistance and evolution to cancer for early stopping criteria in trials.

Clonal evolution is a dynamic, stochastic process

Because somatic evolution is a stochastic process that is dominated by outliers with greatest fitness in the cell population, biomarkers for risk stratification and prediction based on the presence or absence of a phenotypic or molecular lesion in a population of cells are inherently unstable. The clone with the marker may not have evolved yet, it may be a small minority that is difficult to detect, or it may go extinct in the future (Fig. 2). An alternative is to measure the rate of somatic evolution and develop interventions that slow the rate of progression (49). Using high-density SNP arrays, we found evidence for large clonal expansions, clonal extinction, and long periods of relative stasis lasting over 12 years in which the clonal composition of the Barrett’s segment was relatively stable, with the exception of the accumulation of small copy number and LOH lesions (Fig. 2). This case study could be the prototype for large, well-designed studies using a uniform platform to determine the extent to which chromosome instability, clonal expansions, and clonal diversity predict progression to esophageal adenocarcinoma, as well as identifying benign subsets of individuals who require no clinical intervention because of their low risk. Whereas genomic biomarkers characterize somatic alterations in snapshots of time during patient evaluation, clonal evolutionary biomarkers have the potential to summarize temporal changes of genomic state that may be widely applicable to a broad range of neoplasms, because genomic instability, selection of variants, and clonal expansions are believed to occur during progression in most (if not all) cancers.
Characterizing phenotypes of benign and dangerous clonal populations

Although expression and proteomic studies are largely in the discovery phase in Barrett’s esophagus and esophageal adenocarcinoma, their potential to be translated to the clinic to identify a population of individuals who will follow a benign course should not be underestimated, especially in view of the results of the combined expression and proteomic study described above (44). As another example, a recent study of glioblastoma and glioma from the Cancer Genome Atlas Research Network reported that DNA methylation analysis using Illumina Golden Gate and Infinium arrays

Figure 2. Benign clonal evolution in 1 patient with Barrett’s esophagus studied longitudinally over 16 years. Purified Barrett’s epithelium from endoscopic biopsies was assayed with Illumina 317K SNP arrays and compared with a blood sample control. A, Copy number analysis, normalized by SNP intensities from blood, reveals a single copy loss at CDKN2A in samples 2 (data not shown) and 3 in 1989, but homozygous deletion in CDKN2A in sample 1 and all samples from subsequent years. At first endoscopy in 1989, 2 clones were detected (1 with a small deletion of 1 allele at the CDKN2A locus, and the other with copy neutral LOH of the entire 9p arm with the CDKN2A deleted allele, generating biallelic deletion at CDKN2A). B, the SNP allele frequencies reveal a focal deletion in the CDKN2A locus in samples 2 and 3 in 1989, but sample 1 included a mixture of the clone from samples 2 and 3 with a new clone with copy neutral LOH of 9p and biallelic deletion of CDKN2A. All samples from 1993 and later show that the clone with biallelic deletion of CDKN2A went to fixation, leading to random noise in the allele frequencies for the SNPs in that region, as seen in the vertical (“waterfall”) band in the bottom panel of B. The fact that the rest of the 9p arm remains diploid can be seen in the copy number data (A). C, The clone with deletion of the single allele of CDKN2A, which extends past 22.5 Mb on chromosome 9p, also had a single deletion in fragile site FRA3B at 60.42 Mb that distinguishes it from the other clones. This and other lesions of the clone in samples 2 and 3 were not observed again after 1989, suggesting that this clone was driven to extinction by the clone from sample 1, with biallelic deletion of CDKN2A. D, A Camin-Sokal maximum parsimony reconstruction of the genealogy of clones based on the polymorphic copy number of lesions in 283 loci across the entire genome in the Barrett’s biopsies shows that only one large clonal expansion occurred between 1989 and 1993. After 1993, the Barrett’s segment remained stable, with accumulation of small interstitial lesions but no clonal expansions, no aneuploidy, and no progression to cancer.
combined with expression analysis identified a subset of patients with significantly improved prognosis (50). DNA methylation, expression, and protein profiling may be an approach to distinguishing specialized intestinal metaplasia that has adapted to reflux and will remain stable for prolonged periods from a genetically unstable clone with greater risk of esophageal adenocarcinoma.

**Ongoing randomized trials**

A large chemoprevention trial is being conducted in the United Kingdom in individuals with Barrett’s esophagus without high-grade dysplasia, using 2 doses of aspirin (0 vs. 300 mg) with an all-cause mortality endpoint (51). The same trial also includes randomization to low- and high-dose proton pump inhibitor therapy. This trial may provide useful information about the role of aspirin and proton pump therapy for chemoprevention in individuals without high-grade dysplasia in Barrett’s esophagus. A randomized trial of endoscopic surveillance with biopsies every 2 years for about 10 years versus endoscopy for symptoms is in the recruitment stage in the United Kingdom (52).

**Potential game changer: identification of infectious agents that modulate clonal evolution in the distal esophagus**

*Helicobacter pylori* infection has been reported to be associated with an increased risk of gastric adenocarcinoma and decreased risk of esophageal adenocarcinoma (53). Recent research suggests that the microbiome of the distal esophagus is different in health and disease (54). If further research reveals an infectious agent for esophageal adenocarcinoma, the approach to prevention could change dramatically with the potential use of vaccines or antibiotics.

**Conclusions**

New research suggests that specialized intestinal metaplasia contributes to mucosal defense in most individuals, and the vast majority of these individuals will live out their lives unaffected by esophageal adenocarcinoma. However, a small number of individuals in the population develop profound changes in their genomes that lead to esophageal adenocarcinoma. Advances in esophageal genomics and detection of individuals with inherited mutations placing them at risk for esophageal adenocarcinoma will inform increasingly sophisticated risk models to improve identification of patients at risk for esophageal adenocarcinoma, provide opportunity for advances in prevention, and guide selection of interventions appropriate to risk.

**Disclosure of Potential Conflict of Interest**

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