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Genetic Insights in Barrett's Esophagus and Esophageal Adenocarcinoma

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Abstract

Beginning in the 1980s, an alarming rise in the incidence of esophageal adenocarcinoma (EA) led to screening of patients with reflux to detect Barrett's esophagus (BE) and surveillance of BE to detect early EA. This strategy, based on linear progression disease models, resulted in selective detection of BE that does not progress to EA over a lifetime (overdiagnosis) and missed BE that rapidly progresses to EA (underdiagnosis). Here we review the historical thought processes that resulted in this undesired outcome and the transformation in our understanding of genetic and evolutionary principles governing neoplastic progression that has come from application of modern genomic technologies to cancers and their precursors. This new synthesis provides improved strategies for prevention and early detection of EA by addressing the environmental and mutational processes that can determine "windows of opportunity" in time to detect rapidly progressing BE and distinguish it from slowly or non-progressing BE.

Keywords

genomics; chromosome instability; evolution; overdiagnosis

Overview

The challenges facing attempts to reduce mortality of EA by prevention and early detection include overdiagnosis and overtreatment of BE as well as failure to detect the vast majority of EAs when they are early and curable ("underdiagnosis") [1–10]. Overdiagnosis is defined as diagnosis of a disease, typically by screening, that will cause neither symptoms nor death during the lifetime of an individual [11, 12]. A recent review found that 90% of individuals

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with BE die of causes unrelated to EA (overdiagnosis) and that 93% of EAs are not detected by current screening strategies and instead present as advanced, symptomatic EAs with high mortality (underdiagnosis) [13]. Here, we explore the implications of incorporating advances in genome technology with genetic and evolutionary principles into a "new synthesis" of BE and EA. This synthesis will include genetic approaches to cancer and its precursors that can address the challenges of overdiagnosis and underdiagnosis. This review is focused on genetics (the classical science of heredity) and genomics (the comprehensive study of alterations in both the constitutive genome (the level of the individual) and the somatic genome (within cells of the body including neoplasms), and the evolution of neoplastic cell lineages during progression to cancer that can be evaluated by genetic and genomic approaches. These concepts are at the core of many current challenges in cancer control as seen by the clinical gastroenterologist. This review does not address alterations in DNA methylation, chromatin remodeling or proteomics.

Barrett's esophagus, prevention and early detection of esophageal adenocarcinoma

In 1950, Norman Barrett proposed that chronic peptic ulcers of the esophagus arose in a columnar lining resulting from a congenital short esophagus [14]. Three years later, Alison and Johnstone presented a series of case reports calling attention to the association between BE, gastroesophageal reflux and esophageal ulcers and offered the competing but not mutually exclusive hypothesis that the columnar lining resulted from gastric epithelial overgrowth of the ulcer during healing [15]. In 1957, Barrett responded with an insightful manuscript that reviewed comparative anatomy studies indicating that the esophageal squamous lining extends into the anatomic stomach in many animals, including the horse, cow, rat, rabbit and platypus [16]. He also reviewed embryology, citing the Johns manuscript that reported the embryonic human esophagus has a columnar lining at early stages of development before it is replaced by a squamous lining [17], similar to recent reports in mouse models [18]. In 1975, Naef et al. reported a large series of 1225 patients with reflux esophagitis, 140 patients with BE and 12 EAs completing the triad that continues to dominate current clinical thought [19].

Beginning in the 1980s, the incidence of EA began to increase at an alarming rate in the United States and much of the western world [20–23]. Given the associations between gastroesophageal reflux, BE and EA, it therefore seemed evident that the way to control the rising incidence of this deadly cancer would be to screen the population with reflux symptoms by endoscopy to detect BE and monitor those with BE by endoscopic biopsy surveillance for early detection of EA [24]. This clinical response was not unreasonable given the prevailing paradigm of gradual linear progression to cancer that the investigators were taught during medical training [25]. However, these well-intentioned efforts at early detection selectively identified patients in whom BE will cause neither EA, death nor symptoms during their lifetime while failing to detect the vast majority of EAs that continue to present at an advanced stage with poor prognoses [4, 5, 8, 9, 13, 26]. Thus, seemingly paradoxically, these attempts to control EA based on accepted medical concepts resulted in overdiagnosis of benign BE and underdiagnosis of life-threatening EA [13]. To understand the apparently

paradoxical behavior of BE and EA as well as over- and underdiagnosis of many other cancers and “precancerous” conditions, long held clinical beliefs need to be re-evaluated in light of recent genetic and genomic advances.

These apparently paradoxical screening results are believed to be due to length-biased sampling, which postulates that screening tests selectively detect slowly or non-progressive conditions and miss rapidly progressing disease [25] (Figure 1). If this hypothesis is correct, then time and space become two of the most critical variables for early detection. To understand and overcome the challenges posed by length-biased sampling and evolution of resistance and relapse after therapy, recent genomic advances need to be reviewed as they pertain to somatic genomic evolution of cancer. We begin with a short history of medical and genetic theories of neoplastic progression.

Medical and genetic cancer progression theories

The National Cancer Act was passed while the first author of this manuscript was a graduate student in genetics. The department was a hotbed of discussion about a theory that cancer was a disease of branching evolution of somatic genomes in organs and tissues of the body [27]. The author entered medical school to investigate this concept, where he encountered a competing theory. For more than a century, medical training has been based on the concept of gradual, linear progression of disease [25]. This concept was reinforced by linear models that represented neoplastic progression as a gradual accumulation of molecular abnormalities leading to cancer [28, 29] (Figure 2). However, the authors of these papers and others have cautioned that progression would be more complex [30]. For example, more recent studies indicate that somatic genome evolution in cancer may be branched and occur much more rapidly than anticipated [31–35] although there is also evidence that some cancers may evolve gradually [29, 36]. The differences between these two progression models have profound implications for cancer prevention and early detection: Linear models predict that inhibiting a single step will interrupt progression to cancer whereas branched evolution provides avenues for resistance to therapies that target only one branch (Figure 2).

In 1859 Charles Darwin proposed that organisms evolved by natural selection [37]. Shortly thereafter in 1865, Mendel published his work on inheritance in peas, but his genetic studies were lost and only rediscovered in 1900 [38]. In subsequent decades, the “modern synthesis” reconciled Darwin’s theory of evolution with Mendelian genetics as source of hereditary variation on which natural selection acted to promote evolution. The theory of neoplastic evolution predicted that genomic instability would promote branched evolution of cancers, each with a unique somatic genome that might require individualized therapy and carry the potential for evolution of resistance to preventive and therapeutic interventions was proposed by Nowell in 1976 [27]. Yet evolutionary principles have been poorly integrated into medical thought, training and practice. For example, a recent review evaluated 6,228 abstracts on therapeutic resistance and/or relapse and found evolutionary terms were used in only about 1%. Detailed coding of 22 recent papers revealed a higher proportion of use of evolutionary methods or theory, but this was less than 10% [39].

Modern genome sequencing and SNP array technologies have provided the breakthroughs to confirm and extend Nowell's theory of branched evolution that predicts emergence of resistant populations of cells after therapy in contrast to linear models that predict inhibition of any step in progression will block downstream events and cancer [32, 40] (Figure 2). These advances will revolutionize the care of patients with BE and EA by facilitating a "new synthesis" incorporating genetics, genomics and somatic genome evolution that can (1) track neoplastic cell lineages regardless of morphology including both BE and neosquamous epithelium, (2) determine which BE will remain in relative genomic stasis (nonprogressing) while others evolve rapidly to EA, (3) identify environmental carcinogens that cause specific mutation signatures providing a new approach to prevention, and (4) give early warning of resistance or relapse after therapy so the patient's disease may be appropriately treated. Modern genome technologies have also provided evidence that neoplastic evolution may be accelerated by increased mutation rates and even more rapidly by punctuated or catastrophic chromosomal events that may occur in one or a few cell divisions (Figure 3) [35, 41]. This rapid evolution generates genetic and genomic variants (diversity) on which selection can act to promote progression to EA [40, 42]. It is imperative that our approaches to prevention and early detection define "windows of opportunity" in time to detect these rapidly evolving genomes [43, 44].

Three genomes may contribute to cancer evolution: (1) somatic genomes that evolve to cancer in nuclei of cells in organs and tissues of the body, (2) mitochondrial genomes and (3) inherited, constitutive genomes propagated in the germline. Advancing genomic technologies including exome and whole genome sequencing and high density SNP arrays have revealed that EA genomes have very high mutation and chromosome aberration frequencies [45–52]. The inherited constitutive genome may have rare highly penetrant mutations that can be detected in family studies [53–58]. Alternatively, risk may be detected by genome-wide association studies (GWAS) [59, 60, 61]. Below, known contributions of each of these genomes to risk of cancer are reviewed, beginning with the somatic nuclear genome, which is the genome that evolves to cancer.

The ability to study genetic and genomic alterations in BE as it does or does not evolve to EA in space and time provides an opportunity to develop studies that meet best practice standards for biomarker research as well as genetic studies (Table 1).

Genomic Evolution of EA and BE

Overview

Recent studies indicate that the concept of gradual, linear progression with long time intervals for early detection may not apply to many EAs and other cancers that appear to arise by chromosome instability, which has historically been defined as an increased rate of gain or loss of whole chromosomes or large regions of chromosomes [62]. Studies using modern genomic advances in sequencing and SNP arrays have reported rapid "punctuated" and/or "catastrophic" chromosome evolutionary events that can develop in one or a few cell divisions [34, 35, 41, 44, 48] (Figure 3).

All somatic genomic mechanisms leading to increased chromosome or nucleotide evolution may result in rapid generation of cellular diversity within the BE segment on which selection can act to promote rapid somatic genomic evolution [34, 35, 41, 44, 48]. This rapid evolution can lead to shorter "windows of opportunity" for early detection by decreasing time intervals required for progression to EA (Figure 3). In this regard, longitudinal studies of BE in space and time using EA endpoints may provide unique insights that are directly applicable to early detection of other cancers, including breast, lung, colon and ovarian among others that have been reported to evolve through stages of punctuated chromosome instability and catastrophic whole genome doublings in TCGA (The Cancer Genome Atlas) [41]. In fact, nearly 40% of all cancers have been reported to have undergone at least one whole genome doubling[63].

Changes in selective pressures including medical treatments may also lead to rapid evolution when selection favors a minority cell population in the BE segment and the majority population is at a selective disadvantage in the new environment [40, 64]. Reported crypt to crypt variation in BE could provide such a source of variants for resistance to therapeutic interventions [65–67].

Genetic and genomic studies of BE and EA

Although this review focuses on recent evidence available as a result of technology advances, it should never be forgotten that this knowledge base has been built on pioneering studies performed by a large number of investigators prior to the spectacular technological advances that make the current studies possible. Historically, genetic and genomic studies of BE and EA have been focused more on chromosome alterations than mutations because of technology availability. DNA content flow (or image) cytometry [68–73], FISH (*fluorescent in situ hybridization*) [74–80], array comparative genomic hybridization [51, 52, 81–86], and *TP53* analyses [31, 87–96] have all contributed to increasing knowledge of the complexity of chromosome instability in the EA genome and the BE genome as it progresses towards cancer. In general, these founding studies support conclusions of more recent genomic investigations providing a broad base on which to develop new approaches to early detection, prevention and therapy.

In the early days of BE genetic research, mutation studies focused on known genes, such as *TP53* and *CDKN2A* because DNA sequencing technology was limited [97–99]. In a study evaluating a panel of tumor suppressor genes and DNA content abnormalities (tetraploidy, aneuploidy), only the chromosome instability markers, loss of heterozygosity (LOH), tetraploidy and aneuploidy, provided independent cancer risk assessment in multivariate analysis [100]. Interestingly, use of aspirin or other NSAIDs was associated with reduced risk of progression to EA in patients with 17pLOH and DNA content tetraploidy and aneuploidy in this study.

Genomic studies of EA

At the time of writing this manuscript, TCGA study of EA has not been published. The interested reader should certainly review the TCGA study when its results become available because it is likely to be the standard reference for some time. Much has already been

learned from several sequencing and high density SNP array studies that have shown that EAs typically have massive genomic alterations, including high frequencies of mutations and chromosome alterations (see Supplementary Data in [45–49, 52, 101] for comprehensive listing of mutations and copy number alterations detected in EA). However, caution is urged in interpreting cancer-only study designs because, as shown below, common early events that are frequently detected in this type of design can also be found at equal frequency in BE that does not progress to cancer [44]. Basing risk assessment on these frequent, non-progressing alterations can exacerbate overdiagnosis and overtreatment in BE.

DNA sequencing studies of EAs

EAs arise in a highly genotoxic environment in which the distal esophagus is exposed to high levels of local and systemic injury from reflux of acid, bile and other gastric contents, tobacco products, and inflammatory responses to the injury, all of which are mutagenic [2]. EAs have very high mutation frequencies exceeded only by bladder, melanoma, and lung cancer [45, 47, 102]. In the largest study, Dulak et al evaluated 149 normal/tumor pairs by exome sequencing, with 15 also evaluated by whole genome sequencing[47]. The median mutation frequency across the genome per cancer was 26,161 with whole genome sequencing (range 18,881–66,225 mutations per cancer).

The authors also reported a high frequency of AA>AC transversions at AA nucleotides, a mutation signature that has been confirmed by other studies [48]. This signature has been reported only in esophageal and gastric adenocarcinomas [103, 104]. Other more common mutation signatures, such as the APOBEC cytidine deaminase signature and an aging signature, have also been identified [102]. In the Dulak study, 8,331 genes had mutations in at least one EA, but only *TP53* was mutated at high frequency (72%). This ground breaking manuscript reported many mutated genes that had not been previously detected in EAs that were potential targets for therapy, but only *TP53* was mutated at sufficiently high frequency to have a major impact on early detection or prevention. Other, smaller exome sequencing studies and one whole genome sequencing study of 22 patients have also reported that *TP53* is the only commonly mutated gene in EAs [45, 48, 101, 105]. Localized regions of hypermutation (“kataegis”), a BRCA-deficiency signature and a previously unknown signature have also been reported in subsets of EA [48]. These different mutation signatures presumably represent the highly genotoxic environment in which EA arises, but no direct causality has yet been demonstrated for many of them [48].

Chromosome alterations in EAs

EAs have high frequencies of somatic chromosome evolution, including classical chromosome instability, which can occur in a series of “punctuated” events, followed by catastrophic chromosome evolutionary events, including whole genome doublings, which can develop in a single cell division [41, 46–50, 52, 83, 106, 107]. Chromothripsis (“chromosome shattering”) can also develop in one or a few cell divisions [35, 48]. These modern sequencing and SNP array data provide additional support for the concept that cancer evolves through a genome doubling (“tetraploidization”) followed by additional chromosome instability, which has been well recognized in the cytogenetic and flow cytometric literature for several decades [108]. Evidence of chromothripsis was found in 36% of EAs in a recent combined

study of whole genome sequencing (22 EAs) and SNP arrays (101 EAs) [48]. Interestingly, the same study also reported evidence of breakage-fusion-bridge cycles that can develop as a result of telomere attrition [48]. The breakage-fusion-bridge cycle findings are consistent with findings of other investigators that short telomeres in (1) BE are associated with chromosome instability [109], (2) in the blood are a risk factor for progression from BE to EA [110] and (3) are found in EAs themselves [111].

DNA sequencing studies in BE

Less is known about mutations in BE, and much of the knowledge that exists comes from patients who progressed to EA and had co-existing BE that was sequenced [45, 101]. In one study, exome sequencing was performed to evaluate 11 EAs, two of which had matching samples from BE [45]. In one patient, 65 of 78 mutations detected in EA were also found in BE. In the second patient, 31 of 39 mutations detected in EA were also found in adjacent BE [45]. In a second paper, biopsies from a single EA and adjacent BE were evaluated by whole genome sequencing, and the investigators found that the mutational profiles of EA and BE were remarkably similar [101]. However, these case-report studies did not include nonprogressing control populations.

These observations were recently extended in a cross-sectional study that included several components [105]. This study performed whole genome sequencing in a discovery set of 22 EAs. Mutations detected above background rate in the discovery set and in pathways of interest were then validated in a larger set of 90 EAs. Combining mutations found in discovery and validation resulted in only 15 genes that were mutated in four or more samples. Consistent with the results of Dulak et al., the only gene that was mutated at high frequency was *TP53* (69%). Twenty-six genes were then evaluated in a cross-sectional analysis of 66 biopsies from 40 patients who were always negative for dysplasia during follow-up and 43 biopsies from 39 patients who had coexisting high-grade dysplasia. Twenty-one of 40 patients whose biopsies were consistently non-dysplastic (53%) had mutations in the BE segment. The mutational frequency was not significantly different between non-dysplastic BE, high-grade dysplasia and EA; only *TP53* and *SMAD4* were associated with advanced stages of progression, and *SMAD4* was mutated at low frequency in only 13% of EAs. Initial validation of a non-endoscopic screening device (“Cytosponge”) to detect *TP53* mutations in this study is a major step forward toward developing more effective screening strategies for high-risk BE and early EA [105].

Chromosome studies in BE

Genomic studies in BE have consistently reported the presence of chromosome alterations. As the density of markers has increased, small localized regions of copy number alterations and LOH appear to be frequently found in fragile sites some of which contain genes such as *CDKN2A*, *WWOX* and *FHIT* that are deleted frequently [86, 112]. It was initially hypothesized that these findings might be due to chronic reflux exposure and genotoxic stress due to oxidative damage, and stalling at DNA replication forks that merited evaluation as a biomarker of EA risk in patients with BE [112]. There is also evidence that massive and small chromosome alterations can be detected in BE before EA [44, 50, 113]. Early studies using low density STR polymorphisms implicated loss of chromosome arm 9p and *CDKN2A* in

progression to EAs [100]. However, large, well-designed studies have reported that the frequencies of small homozygous deletions in fragile sites involving *CDKN2A*, *FHIT*, and *WWOX* as well as chromosome 9p loss or LOH are not significantly different between progressors to EA and non-progressors [44, 113]. This represents an important principle of early detection and prevention research: well-designed studies with non-progressing control populations are required to distinguish those lesions whose increased risk warrant therapy from benign changes that do not progress. Further research is necessary to characterize the roles of these changes in BE.

The largest longitudinal study of BE using SNP arrays was a case-cohort study of 248 patients with BE of whom 79 progressed to EA while 169 did not [44]. Chromosome alterations, including homozygous deletions, losses, gains, and balanced gains, were assessed in a defined protocol evaluating one endoscopic biopsy by SNP arrays every two centimeters in the Barrett's segment using a constitutive genome control. The patients were evaluated at the baseline endoscopy in the study and the penultimate endoscopy (next to last endoscopy in patients who did not progress to EA or endoscopy before cancer in patients who progressed). Non-progressors largely maintained stable genomes with only minor changes involving fragile sites and small genetic regions including 9p LOH and small deletions and homozygous deletion on 3p, 9p and 16q, the sites of *FHIT*, *CDKN2A*, and *WWOX*. These abnormalities have been also observed at high frequency in non-progressors in previous studies [113]. It has recently been proposed that everyone may develop similar genetic alterations during their lifetimes without progressing to clinically evident cancer [114].

In contrast, massive genomic alterations including widespread evidence of chromosome instability were detected beginning 48 months before the diagnosis of cancer, followed by catastrophic genome doublings and widespread aneuploidization in the 24 months before cancer [44]. Strikingly, this pattern of chromosome instability followed by whole genome doublings has been observed in many types of cancer including esophageal, breast, colon, lung, and ovarian [41, 115]. This sequence is also very similar to a previous report in which 17p LOH was strongly associated with development of increased 4N (G2/tetraploid) populations that were followed by development of aneuploid cell populations in BE approximately 17 months later [116].

Mitochondrial DNA mutations have been reported in EA, BE and cell cultures derived from BE [117–120]. No large scale studies have been reported in either BE or EA, and their role in progression is currently unknown. Mitochondrial mutations have been used innovatively to characterize genetic lineages that assess clonal relationships between Barrett's metaplasia and esophageal squamous cells [67]. Using a combination of DNA sequencing to detect mutations in cytochrome *c* oxidase and immunohistochemistry to detect the enzymatic activity, the authors were able to demonstrate that Barrett's glands were clonal. They also showed that the clonal glands were able to develop all the differentiated cell lineages found in BE. The glands were able to spread forming patches within the epithelium, and in one patient regenerating squamous epithelium and the underlying glandular epithelium shared a clonal mutation establishing that squamous and metaplastic epithelium were derived from a common precursor.

The constitutive genome has been evaluated in family studies and two genome-wide association studies (GWAS). A genetic component to developing reflux, BE and EA has been suspected based on GWAS studies [121], analysis of familial clusters [53–57, 122] and twin studies [123, 124]. A complete family history is recommended for patients being seen for BE or EA [125]. The impact of identification of genetic risk factors on patients and families who are at risk for inherited BE and EA is profound for both patients who inherit the risk and for relatives who do not. Although they represent a small portion of the population and the number of people who develop EA, the benefits of early diagnosis and prevention are great in these patients. Research to identify the genetic loci that account for the increased risk in family studies of BE and EA is ongoing.

Two GWAS studies have been published. One reported that variants at the MHC locus and at chromosome 16q24.1 predispose to development of BE [61]. The closest protein-coding gene to the 16q24.1 locus is *FOXF1*, which has been implicated in esophageal development and structure. The other GWAS identified four associations, including 19p13 in *CRTC1*, which has also been implicated in esophageal development [59]. Other loci identified include one at 9q22 in *BARX1*, which codes for a transcription factor for esophageal specification. A third was at 3p14 near *FOXP1*, which is known to regulate esophageal development. This study also confirmed the previously reported association with BE near *FOXF1* at 16q24, which was also associated with EA. One study reported that some *CDKN2A* polymorphisms were associated with reduced risk of EA [126]. This protective association is reminiscent of reports that *CDKN2A* abnormalities are associated with clonal expansions in BE but the chromosome instability leading to 17p LOH, tetraploidy and aneuploidy is required for progression to EA [64].

Integrative team science, a path forward: “The future ain’t what it used to be.” [127]

It would be a mistake to think of genetics and genomics as simply “biomarkers” because the modern synthesis presaged an era in which genetic and genomic approaches can permit analyses of evolving cell lineages that can track clones over space and time in individual BE segments as well as in human population studies. Team science approaches that incorporate advances in genome technologies combined with application of evolutionary principles of selection of genetic variants will revolutionize the care of patients with BE and EA (Figure 4).

The power of genetic lineage analysis is also illustrated by the seemingly simple experiment described above using DNA sequencing of the mitochondrial genome that found that regenerating squamous epithelium and underlying glandular epithelium were derived from a common precursor [67]. A subsequent somatic genetic study evaluated pre- and post-ablation epithelia in patients with BE [128]. In this study, somatic mutations involving *TP53* and/or *CDKN2A* were found in post-ablation neosquamous epithelium and deep esophageal glands. Non-dysplastic BE epithelium was also found to contain mutant clones that were subsequently found in EA demonstrating a lineage that evolved and progressed over time. There have been at least four case reports of esophageal squamous cell carcinomas arising after various forms of ablation of BE [129–131]. Genomic evaluation of resection margins after endoscopic therapy might therefore be used for detection of residual disease much like

pathology margins are currently used after surgery. Somatic genomic assessment can also be used to monitor squamous and columnar epithelium after ablation therapy.

Although it is not covered in detail in this review of genetics and genomics in BE and EA, it is worth mentioning that recent research by a number of investigators has shown BE intestinal metaplasia has a number of properties that appear to be selective protective adaptations to the harsh, genotoxic environment in which BE arises [2, 132]. Some of these adaptations, such as crypt architecture have long been thought to have evolved as a mechanism to prevent cancer by decreasing clonal expansions of mutations [133]. Observations of specific mutation signatures in EAs may indicate the presence of environmental mutagens against which the protective adaptation might have been lost or never evolved. This could guide prevention strategies for EA by eliminating the environmental mutagen or other risk factors such as obesity [2, 134].

GWAS studies have identified a number of highly intriguing loci that are involved in esophageal development [59, 61]. Recent studies of p63 knockout mice have shown that the embryonic mouse esophagus is lined by a columnar epithelium that is remarkably similar to BE [18]. These studies, combined with rediscovery of the Johns manuscript, will fuel the debate and drive scientific inquiries about the origin of BE [17]. This research suggests that the ability to generate Barrett's metaplasia in response to a reflux environment is the result of a developmental program that could be the target of additional research into the origin of the BE. A better understanding of how the Barrett's epithelium originates could allow development of better screening strategies to identify patients at risk for developing BE in the population in general and potentially screening for high risk BE.

Researchers are searching for inherited highly penetrant mutations that predispose to BE and EA in familial clusters. Identification of the inherited genes will have a profound effect on the families: One sibling may inherit the mutation and a second will be unaffected. They will live different lives, and their parents, physicians and counselors will need to be sensitive to their different needs. Once, long ago when the first author of this paper was in training, a senior attending physician seeing a patient with an inherited susceptibility to a disease said to the patient "You're a mutant". We are entering a world of genomic medicine, and our training programs need to include genome biologists, geneticists and genetic counselors so that future gastroenterologists, who will be involved in the care of these patients, will be attentive to their needs.

Summary

A recent perspective on BE and EA appropriately commented that "The current strategy can be construed as representing not a 'war' on oesophageal adenocarcinoma, but rather a war on Barrett oesophagus" [13] with the unintended consequence of overdiagnosis. Assuming the goal is to reduce mortality of EA, this "war on BE" will fail since the current strategies for early detection result in 90% overdiagnosis of benign BE that causes neither death nor symptoms over a lifetime *and* 93% underdiagnosis of early EA. This strategy can become even more deeply flawed if overdiagnosis leads to overtreatment.

As Ruth Sager said, “Cancer is a disease of the genome.”[135] Multidisciplinary research teams using proper application of genetic and evolutionary principles combined with modern genomic advances can markedly improve our ability to diagnose risk, define windows of opportunity for early detection and guide interventions to prevent EA (Figure 4). Successful incorporation of the genetic, genomic and evolutionary principles described here will lead to a “new synthesis” of BE and EA based on genetic and genomic advances applied in a context of evolutionary dynamics over time and space that reduce both over- and underdiagnosis. Successful research will lead to more accurate identification of patients in whom interventions can prolong functional life by preventing EA.

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Abbreviations

BE	Barrett’s esophagus
EA	esophageal adenocarcinoma
TCGA	The Cancer Genome Atlas

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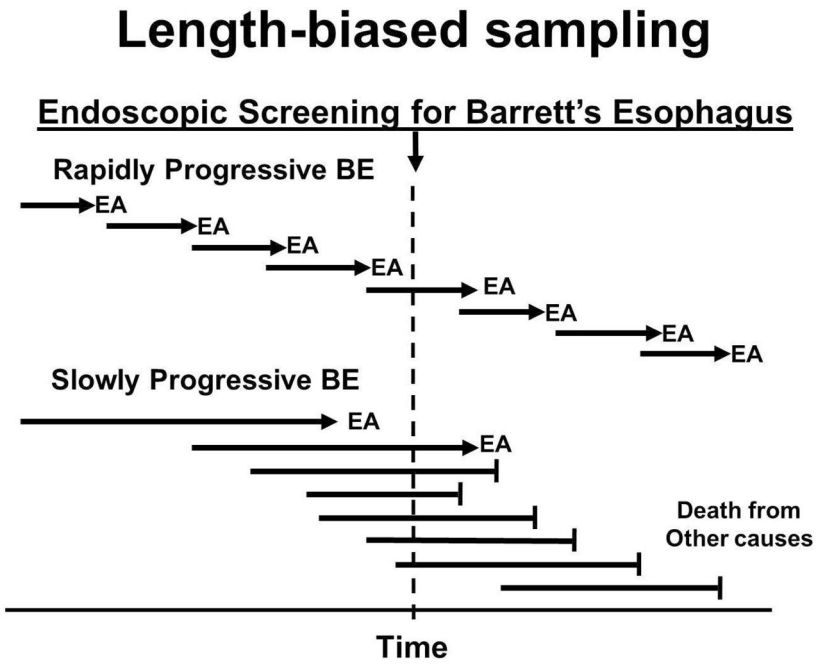

Figure 1. Length-biased sampling

Screening tests tend to be more effective at detecting slowly evolving neoplasms than those that progress rapidly. In some cases, neoplastic evolution occurs so quickly that the patient develops an advanced cancer that was not detected by screening. However, if the disease progresses sufficiently slowly or not at all, the patient can die of unrelated causes ("overdiagnosis"). This is believed to result from length-biased sampling. Research is needed to overcome length-biased sampling in BE screening including (1) identification of the duration of the window of opportunity in time so that screening intervals can be determined to detect rapidly evolving BE before it progresses to an incurable EA and (2) development of biomarkers that distinguish rapidly progressing BE from BE that evolves slowly or not at all.

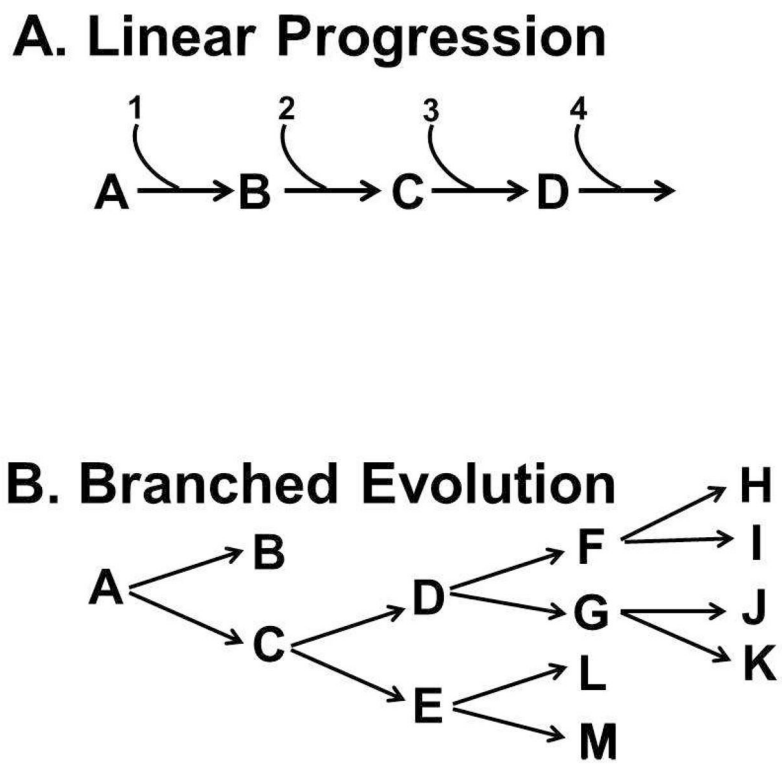

Figure 2. Linear and branched evolution of cancer

Panel A. The linear model of disease has dominated medical thinking about early detection for more than a century. In its recent versions, it has postulated that a slow, gradual linear occurrence of molecular abnormalities (1, 2, 3, 4) cause changes in tissues (A, B, C, D) before the onset of cancer. This model predicts that interrupting any event (e.g., B) in the linear pathway will prevent progression. Panel B. Recent advances in genome technologies have reported that cancers arise by "branched evolution". In some cases, such as in BE, an early branch leads to a state in which the esophageal metaplasia can remain stable for prolong periods of time even though it has some genomic alterations (B). However, in other BE, progression is branched. In this case inhibiting one step (e.g., E) will not necessarily block progression, which can proceed through C→D.

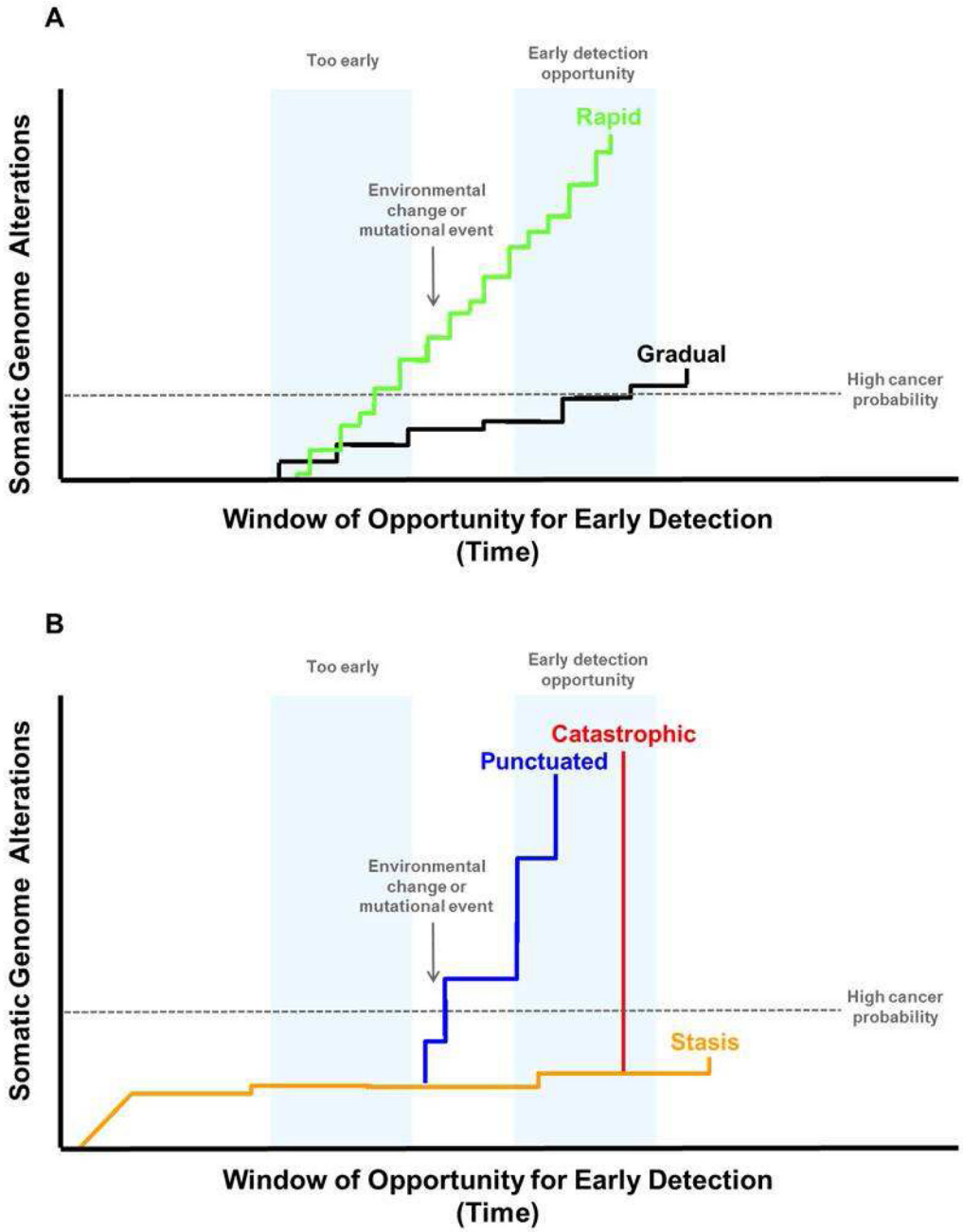

Figure 3. Punctuated and catastrophic evolution can decrease the window of opportunity for early detection

Panel A. Progression of a neoplasm over time has historically been thought to occur by a slow, gradual mutation rate that would typically take decades to accumulate the genetic changes needed to produce a cancer ("gradualism"). However, recent results from genome analyses of advanced cancers have provided evidence that genomic alterations may occur at vastly different rates. For example, point mutations may occur slowly resulting in relatively gradual rates of progression (black line), but exposure to environmental mutagens such as tobacco smoke (lung cancer) and sunlight (melanoma) as well as inherited conditions, such

as mutations that cause microsatellite instability, can increase the mutation rate leading to rapid evolution of cancer (green line). A recent exome sequencing study of a large number of EAs found that MIN was uncommon with only 4/149 that had such high mutation frequencies (2.7%) [47]. Panel B. Chromosome instability, which has been historically defined as an increased rate of gain or loss of whole chromosomes or large regions of chromosomes, causes "punctuated" evolutionary jumps (blue line). Some genomic errors involving chromosomes can lead to catastrophic evolution. These include chromothripsis (chromosome shattering) and whole genome doublings, which may occur in a single cell division (red line). A series of events may accelerate progression from punctuated to catastrophic evolution and cancer. For example, BE develops chromosome instability ("punctuated evolution" blue line) within four years of the diagnosis of EA and genome doublings ("catastrophic evolution" red line) that can be detected by SNP arrays within two years of EA diagnosis (red line). When evaluated by SNP arrays, non-progressing BE typically has a limited number of chromosomal alterations that developed before the patient was seen clinically and tend to remain stable for prolonged periods up to more than two decades ("stasis" yellow).

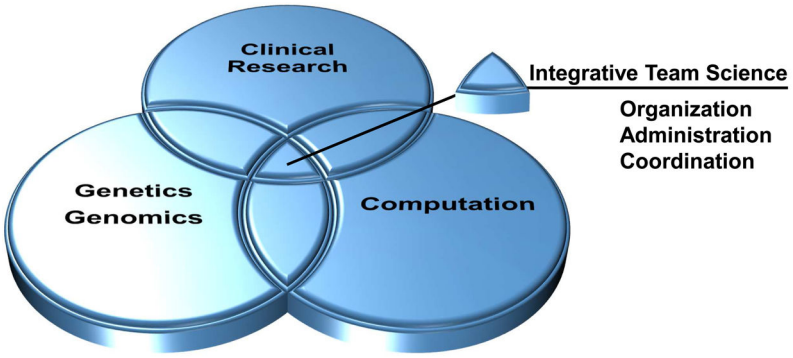

Figure 4. Integrative team science for genetic/genomic studies of BE

BE is a complex adaptive system that can evolve into a stable state that persists for the lifetime of 90–95% of individuals or enter a process of dynamic, stochastic somatic genomic evolution in space and time that leads to EA. The path forward will require multidisciplinary studies that include: (1) Genetics, genomics, and evolutionary biology, (2) advanced computational approaches, (3) clinical and epidemiological research with well annotated biospecimens and (4) integration in an organizational structure with smoothly functioning translational research units. The size of the teams can be variable ranging from two or three collaborators with appropriate expertise to large consortiums. It is likely that success will also require specialized computational support to answer specific clinical research questions. For example, studies of the altered developmental program that leads to BE could be studied at the genomic, expression and/or proteomic levels in BE biopsies and genetic model systems [18, 146]. Alternatively, the program could be investigated in *comparative genomic studies*, which will become increasingly available as more species are sequenced. For example, genes required for acid secretion have been recently found to be mutated or deleted in the platypus[147], which Barrett evaluated in his comparative anatomy studies [16]. The probability of success can be enhanced by increased education and training in genetics, genomics and evolutionary biology as part of GI programs and GI national meetings. The path forward is through collaboration and team science, with each team building the structure required for their specific research hypotheses and questions around institutional strengths.

Table 1

Genetic and evolutionary principles for early detection and prevention biomarkers using genomic data

Best practices for translating genomic and evolutionary alterations. A few cautionary comments are in order about the development and use of somatic genetic and genomic alterations as biomarkers for cancer risk management. Multiple studies have embraced some aspects of the approach outlined here, such as using normal constitutive DNA as a control to be certain that changes are due to genomic alterations in BE, whereas other aspects, such as examining multiple samples obtained at multiple time points, are rarely used. For example, prominent studies from TCGA (in EA and in other cancer types), while accomplishing their goals of generating a valuable catalog of mutations that develop in within tumors, are not well suited for identifying biomarkers of risk progression since they did not examine patients who don't progress to cancer or examine samples at multiple positions in space within the esophagus and/or multiple time points [136, 137]. Incorporating multiple measures of genomic alterations as they evolve in space and time within BE is one of the two greatest and easiest advances that can be made in current BE research. The second greatest need is use of an EA outcome because many BE studies rely upon surrogate dysplasia endpoints. Formal criteria for using surrogate markers in clinical studies have been well described [138, 139]. Surrogate endpoints must be reproducible, accurately represent the true endpoint (EA), and have strong predictive ability to distinguish patients who will progress to cancer from those who will not [138]. The current dysplasia classification system does not meet these criteria because it is not reproducible [140–142], does not accurately represent the true endpoint EA [69, 143, 144], and has highly variable outcomes in predicting future progression to EA [3, 69, 143, 144]. The current practice of normalizing genetic biomarkers to dysplasia grade guarantees that the genetic markers will be just as irreproducible as histopathology when they are used in other centers. Changing this practice is a second advance that, combined with the ability to study genetic and genomic alterations in BE as it does or does not evolve to EA in space and time, can provide a more robust analysis of how the cancer develops and evolves as well as providing more effective use of limited numbers of cancer outcomes [31, 44]. Some problems can begin to be overcome by determining the spatial distribution/evolution of genetic alterations surrounding EAs at the time they are diagnosed [44, 145]. This practice will likely increase as diversity within EAs becomes increasingly recognized as essential for planning therapeutic strategies. Other challenges will likely be overcome as technologies advance to allow robust analysis to be performed on archived FFPE material. Biorepositories of fresh frozen material obtained prospectively are very rare, but biopsies taken for histologic assessment may be repurposed to allow analysis of the evolution of EA over space and time in a larger set of patients. Where applicable, we have included in Table 1 examples of studies that illustrate use of these principles.

1	Base genomic biomarkers on fundamental genetic and evolutionary principles [44, 105]
2	Use normal constitutive DNA as a control [45, 47]
3	Well-accepted study designs including patients who do and do not progress to EA [44, 105]
4	Track genomic evolution in esophageal space [45, 65, 101]
5	Track genomic evolution in time [44, 116]
6	Use EA endpoints [44, 143, 144]
7	Be aware of accepted standards for surrogate endpoints [139, 148, 149]
8	Avoid surrogate endpoints that do not meet accepted standards [2]