Temporal and Spatial Evolution of Somatic Chromosomal Alterations: A Case-Cohort Study of Barrett’s Esophagus

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Abstract

All cancers are believed to arise by dynamic, stochastic somatic genomic evolution with genome instability, generation of diversity, and selection of genomic alterations that underlie multistage progression to cancer. Advanced esophageal adenocarcinomas have high levels of somatic copy number alterations. Barrett’s esophagus is a risk factor for developing esophageal adenocarcinoma, and somatic chromosomal alterations (SCA) are known to occur in Barrett’s esophagus. The vast majority (~95%) of individuals with Barrett’s esophagus do not progress to esophageal adenocarcinoma during their lifetimes, but a subset develop esophageal adenocarcinoma, many of which arise rapidly even in carefully monitored patients without visible endoscopic abnormalities at the index endoscopy. Using a well-designed, longitudinal case-cohort study, we characterized SCA as assessed by single-nucleotide polymorphism arrays over space and time in 79 "progressors" with Barrett’s esophagus as they approach the diagnosis of cancer and 169 "nonprogressors" with Barrett’s esophagus who did not progress to esophageal adenocarcinoma over more than 20,425 person-months of follow-up. The genomes of nonprogressors typically had small localized deletions involving fragile sites and 3p loss/copy neutral LOH that generate little genetic diversity and remained relatively stable over prolonged follow-up. As progressors approach the diagnosis of cancer, their genomes developed chromosome instability with initial gains and losses, genomic diversity, and selection of SCAs followed by catastrophic genome doublings. Our results support a model of differential disease dynamics in which nonprogressor genomes largely remain stable over prolonged periods, whereas progressor genomes evolve significantly increased SCA and diversity within four years of esophageal adenocarcinoma diagnosis, suggesting a window of opportunity for early detection. Cancer Prev Res; 7(1); 114–27. ©2013 AACR.

Introduction

The incidence and mortality of esophageal adenocarcinoma have been increasing at an alarming rate over the past 4 decades (1). Esophageal adenocarcinoma typically presents as a symptomatic, highly aggressive, lethal cancer with a low cure rate (2). Even 15% of small superficial submucosal T1b tumors are lethal, which has been attributed to early lymphatic invasion and metastasis (3–5). This dismal prognosis has led to attempts to detect intramucosal esophageal adenocarcinoma, a stage that is nearly 100% curable, by screening and surveillance of Barrett’s esophagus, the only known precursor of esophageal adenocarcinoma (6). However, advanced esophageal adenocarcinomas have been reported to arise even in carefully monitored patients with Barrett’s esophagus without visible endoscopic abnormalities at the index endoscopy (7–9), and most esophageal adenocarcinomas are detected within 3 years of the index endoscopy as reported in multiple population and clinical cohort studies (9–13). In the surveillance study with the highest number of esophageal adenocarcinoma outcomes, 96% of esophageal adenocarcinomas were detected at a highly curable intramucosal stage, but such early detection required a mean of 163 four-quadrant biopsies obtained every 1 cm throughout the Barrett’s esophagus segment without visible evidence of cancer during periodic surveillance (7). The sudden appearance of advanced esophageal adenocarcinomas, many with lymphatic invasion, after...
diagnosis of Barrett’s esophagus in cohort studies despite careful surveillance, combined with evidence that the great majority (90–95%) of patients with Barrett’s esophagus die of causes unrelated to the esophagus, has led experts to question the benefit of screening and surveillance for Barrett’s esophagus (12, 14, 15).

Cancer is a disease of dynamic, stochastic somatic genomic evolution (16, 17). All cancers are believed to arise as a result of (i) somatic genomic instability, (ii) generation of somatic genomic diversity, and (iii) natural selection of variants that underlie progression to cancer (16). Based largely on studies of advanced cancers at a single point in time, it has been inferred that different types of genomic instability have different rates of progression. For example, colorectal and some other cancers are believed to develop through gradual accumulation of point mutations at a normal mutation rate over decades (18, 19), which would provide a prolonged time window of opportunity for early detection. However, many cancers show manifestations of chromosome instability (CIN), which has been defined as an increased rate of gains or losses of whole chromosomes or large regions of chromosomes (17, 20, 21). Recent studies of advanced cancers at single points in time have inferred that somatic chromosome evolution may occur suddenly, perhaps in one or a few cell divisions (22–25), which could greatly accelerate progression to cancer. Such rapid somatic genomic evolution, which has been called “punctuated evolution,” could have profound implications for early detection of esophageal adenocarcinoma and other cancers by narrowing the time window of opportunity for screening and surveillance. Esophageal adenocarcinomas have high levels of somatic chromosome copy number alterations compared with other cancers such as gastric and colorectal (26, 27), and there is evidence that chromosome abnormalities develop in Barrett’s esophagus before esophageal adenocarcinoma (28–30). This high level of genome derangement is believed to be because of exposure of Barrett’s esophagus to gastroduodenal reflux of bile acids, nitrosamines, reactive oxygen species, and inflammatory responses to the injury that are genotoxic, as reviewed in Reid and colleagues (17). Yet, most Barrett’s esophagus do not progress to esophageal adenocarcinoma (12, 17).

Much of the focus of cancer genomic research has understandably been on genes and gene pathways in advanced cancers to improve patient treatment strategies (31, 32). However, relatively little investigation has been devoted to understanding the dynamics of somatic genomic evolution that leads to cancer and underlies clinical observations that some Barrett’s esophagus rapidly progress to esophageal adenocarcinoma whereas the great majority do not progress over a lifetime. Because endoscopic surveillance for early detection has been a standard of care for Barrett’s esophagus for more than 2 decades, it provides an opportunity to investigate somatic genomic evolution over space and time in patients who do and do not progress to esophageal adenocarcinoma. Here we present a study of dynamic somatic evolution in Barrett’s esophagus using a case-cohort design, which unlike case-control or nested case-control studies preserves the characteristics of the entire cohort, allowing evaluation of the temporal occurrence of somatic chromosomal alterations (SCA) and esophageal adenocarcinoma outcomes while permitting a cost-effective approach for genomic investigations (33).

Materials and Methods

Human subjects

The Seattle Barrett’s Esophagus Study has been approved by the University of Washington Human Subjects Review Committee since 1983 with reciprocity from the Fred Hutchinson Cancer Research Center (FHCRC) Institutional Review Board since 1994. FH CRC has an approved Federal Wide Assurance (#FWA00019200) with the Department of Health and Human Services. The IRB Registration number is IRB00005619. Five hundred and sixteen patients with Barrett’s esophagus without esophageal adenocarcinoma at the baseline endoscopy met the inclusion criteria for the study and were placed into periodic endoscopic biopsy surveillance for early detection of esophageal adenocarcinoma. Endoscopic biopsies were obtained using a jumbo forceps and a four-quadrant biopsy protocol for histology and research purposes every 1 to 2 cm intervals along the entire length of the Barrett’s esophagus (34). Histologic evaluation for diagnosis of cancer was performed by an expert gastrointestinal pathologist. Blood samples collected at endoscopy were processed for use as constitutive genome controls.

Study design

The Seattle Barrett’s Esophagus Study is a dynamic cohort study (35). For this study, eligible participants (n = 516) included all cohort members who were enrolled between August 3, 1988 and March 25, 2009 with at least 2 endoscopic visits and sufficient tissue for analyses at each time point. No eligible participants were excluded. We then performed a case-cohort study (33) of 248 patients, including all 79 who progressed to esophageal adenocarcinoma (progressors) and 169 who did not progress during surveillance follow-up (nonprogressors). A random sample of 197 patients from the eligible cohort (Supplementary Table S1) was initially selected regardless of follow-up time. All remaining cases (progressors) who were not initially selected were then added to the study group giving a total of 248 patients who were followed for 20,425 person-months. Thus, the case-cohort study included all research participants who developed cancer by the end of follow-up (March 25, 2009) according to the case-cohort design. No patients were lost because of testing failures. This study design is cost effective as it preserves the characteristics of the cohort while assaying samples with relatively expensive single-nucleotide polymorphism (SNP) arrays from only a subset of individuals. Samples from 2 endoscopies were evaluated per individual. For progressors, these were the biopsies from the baseline endoscopy and the penultimate endoscopy just before the diagnosis of esophageal adenocarcinoma (n = 63) or at the time of diagnosis of esophageal
adenocarcinoma (n = 16). For nonprogressors, the baseline and the endoscopy before the final endoscopy were used.

Sample processing

Previous studies have shown that esophageal adenocarcinomas arise in fields of genomic abnormalities that are larger than the cancers themselves and can be detected using one biopsy every 2 cm in the Barrett's esophagus segment (36-39). One research biopsy obtained at each 2-cm interval from flat mucosa without visible evidence of esophageal adenocarcinoma in the Barrett's esophagus segment was evaluated by SNP arrays to assure uniform, unbiased sampling of Barrett's epithelium (Supplementary Fig. S1A). The mean number of biopsies per endoscopy evaluated by 1 M SNP arrays was 2.4 (range 1–9) in non-progressors and 3.1 (range 1–9) in progressors. The number of biopsies reflects a 2 cm sampling protocol based on varying segment length. Mean segment length was 5.2 cm (range 1–20 cm) in nonprogressors and 7.0 cm (range 0–19 cm) in progressors, consistent with previous results indicating a trend for increased progression with increased segment length (40). Biopsies were collected in MEM with 10% dimethyl sulfoxide, 5% fetal calf serum, 5 mmol/L Hapes, and frozen at −70°C. Biopsies were incubated 45 minutes in 30 mmol/L EDTA in Hanks balanced salt solution (HBSS) at room temperature and the epithelium was isolated by peeling the epithelium away from the underlying stroma (Supplementary Fig. S1B; ref. 41). DNA was extracted using Puregene DNA Isolation Kit (Gentra Systems, Inc.) and quantitated with Picogreen (Quant-iT dsDNA Assay; Invitrogen). DNA from 177 blood samples and 71 gastric biopsies were plated as constitutive controls. Eight samples failed resulting in 1,272 Barrett's esophagus biopsies with a genotype call rate 99.4%. Genotyping was performed at the FHCRC Genomics Laboratory using 200 ng on the Illumina HumanOmni1-Quad beadchip utilizing Infinium HD Super Assay methods, scanned using an Illumina iScan and intensity data extracted with software (GenomeStudio v2010.2 with Genotyping Module v1.7) with a GenCall cutoff of 0.15. Input reports were generated using the Partek Report Plug-in v2.13.1.

HumanOmni1-Quad v1.0 DNA analysis BeadChip SNP array data processing and SCA calling

GenomeStudio was used to calculate chromosome copy number (log R ratio) of each Barrett's esophagus sample paired with its constitutive control and Partek (v6.5) software was used for allele-specific copy number estimation. Allele-specific copy number, b-allele frequency, and flow cytometric ploidy data (available from previously utilized biopsies in 239 individuals from a previous study; ref. 37) were used to inform manual copy number baseline adjustment in 38 of 1,272 samples (Supplementary Fig. S2). All samples then underwent segmentation analysis using Partek (v6.5). Additional methods details are provided in Supplementary Materials.

Genetic divergence to assess spatial dynamics in time windows

To determine if heterogeneity of SCA between samples increases with physical distance in the esophagus in different time windows (0–24, 24–48, and >48 months), we measured genetic divergence (42) to evaluate differences in SCA between all biopsies within an endoscopy for each individual. Genetic divergence is defined as the proportion of 1 Mb genomic segments across the genome with different SCA calls between any 2 comparison biopsies. The higher the value the more divergent are the SCA patterns between the 2 comparison samples. Kruskal–Wallis test was used to compare divergence in each spatial category (2 cm, 4 cm, 6 cm, and >8 cm apart) between nonprogressors and progressors. Mann–Kendall trend test was used to test for a trend of divergence to increase with physical distance within each time window.

Temporal dynamics of SCA

Using SCA data from progressor and nonprogressor biopsies, we assessed odds ratios (OR) using genome-wide SCA during evolution to esophageal adenocarcinoma in each of the 3 time windows (0–24, 24–48, and >48 months). Specifically, in each time window, the OR of SCA in each 1 Mb genomic segment along the whole genome for each type of SCA (yes/no binary variable) was calculated. The P-value for significance of OR was adjusted for multiple comparisons using a false discovery threshold such that the number of falsely discovered 1 Mb genomic segments is 1 or less. The case-cohort study design allowed further quantification of the risk of evolution to esophageal adenocarcinoma for various types of SCA across the genome with consideration of individual patient follow-up time. Specifically, the hazard ratio (HR) of SCA in each 1 Mb genomic segment along the whole genome for each type of SCA (yes/no binary variable) was estimated based on the case-cohort design (43). P-values were adjusted for multiple comparisons at q = 0.01 level. The HR quantified the cancer risk for a group that had a specific SCA versus another group that did not have the same SCA. Thus, a value equal to 1 indicated a particular region of SCA had no altered risk of esophageal adenocarcinoma; a value larger than 1 indicated the SCA had an increased risk for progression to esophageal adenocarcinoma. Therefore, the HR estimated for a specific location of SCA is the risk for cancer development estimated for those who carry the SCA relative to those who do not carry the SCA in the same location.

Results

Global assessment of dynamic evolution of SCA in nonprogressor and progressor populations

Using a unique longitudinal case-cohort study of individuals with Barrett’s esophagus, we measured levels and timing of SCA in nonprogressor and progressor populations as they approach the final endoscopy in the study or the diagnosis of a surveillance-detected cancer, respectively. The case-cohort study design, which is described
in detail in methods, considers the temporal relationship of SCA and esophageal adenocarcinoma outcomes and preserves the characteristics of the entire cohort (Supplementary Table S1; refs. 33 and 43). To ascertain an unbiased temporal and spatial sampling from progressors and non-progressors, we analyzed one biopsy from endoscopically unremarkable Barrett’s esophagus mucosa without visible evidence of cancer obtained from every 2 cm interval of the Barrett’s esophagus segment from 2 endoscopies in each individual, a baseline endoscopic biopsy procedure and the procedure before their final endoscopy (169 non-progressors) or their last endoscopy before or at the time of cancer diagnosis (79 progressors). Epithelium isolated from 1,272 mapped esophageal biopsies from the Barrett’s segment were evaluated using Illumina 1 M SNP arrays and compared with normal constitutive controls.

We first assessed total megabases (Mb) of SCA per genome as a metric of whole genome integrity in each biopsy including homozygous deletions, copy loss, copy neutral loss of heterozygosity (cnLOH), balanced copy gain of both alleles, and allele-specific copy gain, as a global measure of SCA over time in the population of progressors and non-progressors as they approach the diagnosis of esophageal adenocarcinoma or the last endoscopy in the study (Fig. 1A–D). Almost all nonprogressor biopsies have SCA by 1 M SNP arrays, but biopsies from nonprogressors had low levels of total SCA that remained low (95% <283 Mb) over prolonged follow-up (range = 0.14–21.4 years, mean = 8.6 years; Fig. 1A and C).

Beyond 48 months, the population of progressors had slightly increased SCA compared with nonprogressors (mean SCA per biopsy = 299.7 and 178.6 Mb, respectively, P = 0.007). In the 24- to 48-month time window, the population of progressors had markedly increased SCA compared with nonprogressors (474.9 Mb vs. 136.8 Mb; P = 7.9 × 10⁻⁶), and this difference became even more pronounced within 0 to 24 months (602.5 Mb vs. 179.8 Mb; P = 5.4 × 10⁻⁸). A similar result was obtained when using percentage of abnormal markers among all the covered markers on the SNP array (Supplementary Table S2) and when time was divided into 12-month windows. In the 0- to 24-month time window, there is a highly significant gap in SCA with no intermediates (P = 0.0049; Monte Carlo simulation test). These populations include biopsies with

![Figure 1. Total SCA dynamics in nonprogressor and progressor populations over time. A, B, total SCA in megabases (Mb; y-axis) per biopsies obtained at times (x-axis) before the last endoscopy in nonprogressors (A) or before diagnosis of esophageal adenocarcinoma (EA) in progressors (B). Solid black lines = trend in mean SCA, dotted black lines = 95% CI of the means fitted by quadratic polynomial regression. Six black points right of (B) are SCA levels from advanced EA surgical resection specimens, with 95% CI of the mean. C, in nonprogressors, many biopsies 105/799 (13%) have <1 Mb SCA per biopsy, and 759/799 (95%) of all nonprogressors biopsies have SCA <283 Mb, indicating that somatic genomic alterations are present in most Barrett’s biopsies but the level of SCA is generally low in nonprogressors. All 799 nonprogressors biopsies are below 1,300 Mb SCA. D, in contrast, only 28/473 (6%) progressor biopsies have <1 Mb SCA, and 168/473 (36%) of biopsies sampled before EA in progressors have SCA above 283 Mb (D). There is a significant gap between 1,300 and 1,850 Mb, then in progressors we see 43/473 (9%) biopsies above 1,850 Mb SCA, all with significant amounts of copy gain, balanced gain, and cnLOH indicative of genome doubling.](#)
up to ~1,300 Mb SCA and biopsies with very high SCA > ~1,850 Mb with evidence of genome doubling not found in nonprogressors (Fig. 1B and D). Because this study was part of a program of early detection, surveillance-detected cancers arising in the progressors were too small to be sampled for array analysis (7). Therefore, we examined SCA levels in 6 surgical resection specimens from patients with advanced symptomatic esophageal adenocarcinoma who were not part of our program of early detection, all of which showed these strikingly high levels of SCA (right side of Fig. 1B).

As a population, nonprogressors have abnormal genomes, but the overall spectrum of SCA remains qualitatively similar across all time windows (Fig. 2A and Supplementary Fig. S3A–S3C). Progressors develop in the genetic background that characterizes nonprogressors, and therefore frequently create mosaic Barrett’s esophagus segments in which some areas of the esophagus have low SCA similar to nonprogressors and others have large-scale genomic alterations (Fig. 2B and Supplementary Fig. S3D–S3F).

Evolution of genetic diversity in nonprogressors and progressors over space and time

Genomic instability generates diverse cellular populations on which selection acts to promote cancer (16). Our previous investigations have been limited to measuring

![Figure 2](image-url)
genetic diversity at a single point in time using low-density microsatellite repeats on 2 chromosomes and DNA content flow cytometry (44, 45), but how diversity evolves over space and time in patients with Barrett’s esophagus who do and do not progress to esophageal adenocarcinoma is unknown. Here, we report genetic divergence over space in the esophagus at different time windows in nonprogressors and progressors (Fig. 3A–F) and in individuals over time (Fig. 4). In the population of nonprogressors, patterns of SCA remain similar between biopsies regardless of their spatial relationship in the esophagus and do not significantly change in any of the time windows (Fig. 3A–C). Before 48 months, there was no significant difference in diversity between progressors and nonprogressors (Fig. 3A and D). In the 24- to 48-month time window before cancer, progressors became more divergent over space in the Barrett’s esophagus segment (Fig. 3E), whereas within 24 months of the diagnosis of esophageal adenocarcinoma divergence is high regardless of physical distance within the Barrett’s esophagus segment (Fig. 3F). To test if there was an effect of Barrett’s esophagus segment length on genetic diversity, we evaluated diversity between adjacent biopsies in short (<3 cm), medium (3–6 cm), and long (>6 cm) Barrett’s esophagus segments. We found no significant difference in genetic diversity in biopsies separated by 2 cm between these groups across all time windows in either nonprogressors or progressors (data not shown).

We then evaluated maximum pairwise divergence between all pairs of spatially separated biopsies within the same endoscopy in all individuals who have at least 2 biopsies in both a baseline and last endoscopy (104 nonprogressors and 57 progressors). Nonprogressors have relatively homogenous genomes that remain comparatively stable over long periods of time with only a small minority having any 2 biopsies at a single timepoint with different SCA calls in more than 15% of 1 Mb genomic segments throughout the genome (Fig. 4A, nonprogressors). In contrast, 32 of 57 (56%) of progressors have biopsies within the same timepoint with different SCA in >15% of the genome, and 14 of 57 (25%) have different SCA patterns in more than 40% of the genome (Fig. 4A, progressors). Typical nonprogressor genomes have a small number of SCA events that expand to most of the Barrett’s esophagus segment before the baseline endoscopy with only small, random events arising in one or a few biopsies over time (Fig. 4B; Circos IDs 451 and 611). Many progressors show high levels of diversity over space throughout the esophagus that does not significantly change over time (Fig. 4B, Circos ID 163) or increases over time (Fig. 4B, ID 772). In a subset of cases, there is a reduction in diversity over time as large genomic changes are selected and expand throughout the esophagus.

Temporal dynamics of SCA
At times, >48 months before the final endoscopy or esophageal adenocarcinoma, both nonprogressors and progressors have similar frequency of SCA involving ~12% of the genome (Fig. 5A; OR > 48 months; Supplementary Table S3). This Barrett’s esophagus SCA landscape includes various regions of small homo- and hemizygous deletions often at known fragile sites and 9p arm loss or cnLOH (Supplementary Table S3), and a low frequency of larger gains on chromosomes 8 and 18. Many of the SCA shared between nonprogressors and progressors are also evident at time windows closer to esophageal adenocarcinoma (Fig. 5B and C and Supplementary Table S3). No regions of SCA were detected in any time window that had a significantly higher frequency of SCA in nonprogressors. These results identify regions of SCA that are common across time in both nonprogressors and progressors.

In contrast, progressors evolved additional SCAs that were not shared with nonprogressors over time. Two small losses on chromosome 18q were the only regions with statistically significantly higher frequency of SCA in progressors at >48 months before esophageal adenocarcinoma (Fig. 5A, green and Supplementary Table S4). Within 24 to 48 months of esophageal adenocarcinoma, ~8% of the genome had developed larger regions of copy loss and gain at increased frequency in progressors, including gains on chromosome 8 and 15q and losses on 5q, 17p, and 18 (Fig. 5B; OR = 24–48 mo; Supplementary Table S4). Within 24 months of esophageal adenocarcinoma diagnosis, we observed genome doublings with extensive balanced gains, cnLOH and gains involving more than the 90% of the genome showing statistically higher frequencies of SCA in progressors (Fig. 5C; OR < 24 mo; Supplementary Tables S4). However, the majority of these genome-wide events were detected at a relatively low frequency of 10% to 20% in the population of progressors. We also observed some events that seemed to be coselected within individual biopsies beginning in the 24- to 48-month time window and continuing in the <1,500 Mb SCA population in the 0- to 24-month time window that were not dominated by the genome doublings (Supplementary Fig. S4). These results provide a timescale on which to anchor multistage neoplastic evolution that distinguishes those in the population with Barrett’s esophagus who progress to esophageal adenocarcinoma from those who do not.

The case-cohort study design takes into account the temporal relationship of SCA and esophageal adenocarcinoma outcomes within individuals in the cohort (33, 43) and allowed quantitative mapping of esophageal adenocarcinoma risk of different SCA types by estimating HR genome-wide (Fig. 5D). Considering follow-up times for each individual, progressors had varying low frequencies of SCA with high HRs across large segments of the genome. Much of this SCA occurred significantly more often or nearly exclusively in progressors in biopsies with genome doublings that resulted in large regions of the genome being potent discriminators of esophageal adenocarcinoma risk. Thus, when genomic instability and diversity were high, we detected a large number of infrequent alterations that are found almost entirely in progressors and thus represent low-frequency, high-risk alterations.
Figure 3. Evolution of genetic diversity over space and time in progressors and nonprogressors. Research biopsies used for this study were obtained according to a systematic predetermined protocol with one biopsy every 2 cm in the Barrett’s segment without any visible evidence of cancer. The top left example shows all pairwise divergence comparisons between biopsies. Comparisons include four 2 cm (green), three 4 cm (blue), two 6 cm (brown) and one 8 cm (black) in the example shown. A–F, population-level genetic divergence of spatially separated biopsies in each endoscopy within time windows in nonprogressors before the final endoscopy (A–C) and in progressors before or at diagnosis of EA (D–F). For each individual, the proportion of 1 Mb genomic segments with a different SCA call (present or absent) was calculated for all possible paired biopsy comparisons at each endoscopy. The X-axis is the percent of 1 Mb genomic segments with different SCA calls between any 2 biopsies, for all pairwise comparisons within an endoscopy for each individual and reflects the distribution of the level of heterogeneity among all pairs of biopsies within an individual. The Y-axis is the physical spatial distance between biopsies. For each time window, divergence between biopsies in each spatial category (2, 4, 6, and 8 cm apart) was compared. In the >48-month time window there was no significant difference in divergence between nonprogressors and progressors in any spatial category ($P > 0.313$ among all 4 comparisons, Kruskal–Wallis test, Fig 3A vs. Fig. 3D). In contrast, for all spatial categories in both the 24–48 and <24-month time windows, progressor biopsies had significantly higher divergence than in nonprogressors ($P < 0.0014$ among all 8 comparisons, Kruskal–Wallis test; B vs. E and C vs. F). In nonprogressors, there was no trend for increasing SCA divergence between biopsies regardless of physical distance apart in the esophagus in >48, 24–48, and <24-month time windows ($P = 0.149$, 0.692, and 0.222, respectively, Mann–Kendall trend test; A–C). Similarly, in progressors, divergence remains low in the >48-month time window regardless of physical distance ($P = 0.26$, Mann–Kendall trend test; D). In contrast, in progressors, the 24- to 48-month time window had a significant trend of increasing divergence with increasing physical distance ($P < 0.0051$, Mann–Kendall trend test; E), with divergence remaining equally high in all physical distance categories in the <24-month time window ($P = 0.279$, Mann–Kendall trend test; F).
Discussion

Endoscopic screening for, and surveillance of, Barrett’s esophagus is recommended by professional guidelines (46). Yet, multiple studies from academic and community practices have consistently reported near-total failure of screening and surveillance of Barrett’s esophagus to reduce the mortality of esophageal adenocarcinoma (14, 17, 47). Here, we address a fundamental property of neoplastic evolution that is rarely assessed: the window of opportunity for detection of genomic abnormalities that will evolve to esophageal adenocarcinoma.

Barrett’s esophagus and esophageal adenocarcinoma arise in a highly genotoxic reflux environment, and both have been reported to have SCA detectable by SNP arrays (26, 28–30, 48). Yet most Barrett’s esophagus do not progress to esophageal adenocarcinoma (12, 14, 15). Here, we present a longitudinal case-cohort study of Barrett’s esophagus that permits an innovative investigation of somatic chromosomal evolution over space and time in a cohort of patients who do and do not progress to esophageal adenocarcinoma. As the population of individuals with Barrett’s esophagus approach the diagnosis of cancer or the final endoscopy in this study, somatic genomes of...
nonprogressors remain relatively stable throughout the Barrett’s segment during long-term follow-up, whereas progressors developed chromosome instability, genomic diversity, selection, and coselection of SCA and genome doublings that result in rapid progression to esophageal adenocarcinoma, primarily within a 4-year time interval before cancer detection. Because our case-cohort study design preserves the characteristics of the entire cohort, we were able to investigate SCA from patients who have different follow-up times based on their rates of neoplastic evolution.

Our spatial and temporal data support a model of non-linear dynamic chromosomal evolution in Barrett’s esophagus (Fig. 6; ref. 49). Nonprogressor somatic genomes were characterized by low levels of SCA, including deletions at fragile sites most commonly involving FHIT and WWOX, localized CDKN2A hemi- and homozygous deletions, and 9p arm loss and cnLOH; events also frequently detected in Barrett’s esophagus and many cancers including esophageal adenocarcinoma (27, 29, 50). These changes seem to have originated in an initial Barrett’s esophagus expansion that occurred before the baseline endoscopy in which Barrett’s epithelium has a selective advantage over squamous epithelium in the harsh, mutagenic environment of acid, and bile reflux (17, 51, 52). Importantly, the somatic genomes of nonprogressors remain remarkably stable across space and time with relative homogeneity of SCA at the level of resolution of 1 M SNP arrays.

Progressors have evidence of accelerating genomic alterations that occur on the background of the Barrett’s esophagus SCA landscape beginning with an initial phase more than 48 months before esophageal adenocarcinoma.
diagnosis characterized by an increased background of gains or losses of whole chromosomes or chromosome arms, although only 18q loss is consistently detected at a higher frequency in progressors. This region includes more than 136 genes, but only SMAD4 was found to be significantly mutated in a recent large whole exome sequencing study of 149 esophageal adenocarcinomas (32). This increase in CIN is followed by increasing genetic diversity over space in the Barrett’s esophagus segment and selection of whole chromosome and chromosomal arm gains and losses that are associated with increased risk of progression in the 24- to 48-month time window before diagnosis of esophageal adenocarcinoma. This was followed by sudden catastrophic genome doublings and high genomic diversity that were detected within 24 months of the diagnosis of surveillance detected esophageal adenocarcinoma. Some progressors had low SCA at the baseline endoscopy but either developed high SCA after baseline or had a small number of somatic alterations that carry high HRs for progression to cancer. Although CIN and genome doublings have been well documented in the majority of esophageal adenocarcinomas, some esophageal adenocarcinomas may develop by other mechanisms; for example, microsatellite instability is believed to underlie the development of a small subset of esophageal adenocarcinomas (32). A subset of low SCA progressors did not undergo the initial expansion of Barrett’s epithelium and presented as short segment Barrett’s esophagus. These may represent a subclass of cancers similar to recently reported copy-number devoid breast cancers (53). Alternatively, it is possible that in short-segment Barrett’s esophagus with little opportunity for clonal expansion, genomic abnormalities could not be detected because they were removed in biopsies taken for clinical management, which are taken before research biopsies for ethical considerations.

Our results are supported by a recent cross-sectional study of advanced cancers by Carter and colleagues, who reported that approximately 70% of advanced esophageal adenocarcinomas had evidence of one or more genome doublings and that these doublings seemed to be preceded by specific arm-level copy-number alterations (54). Their computational simulations showed that the genome doublings could not readily be explained as multiple sequential events on different chromosomes but were likely single doubling events; however, their study design could not determine whether these doublings arose before or after the onset of cancer. Further evidence for this concept is provided by earlier DNA content flow cytometry studies in Barrett’s esophagus reporting that increased 4N fractions are an unstable intermediate that develop in cells with 17p LOH and are followed rapidly by progression to aneuploidy detected on average 17 months later (55). Thus, the evidence in several studies support the concept that genome doublings arise by a single or very few episodes of doubling that occur suddenly rather than gradually over time and have the potential to rapidly evolve to cancer. Somatic evolutionary dynamics of progression, including genomic

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**Figure 6.** Model of somatic evolutionary dynamics of SCA in nonprogressors and progressors. Somatic chromosomal evolution in nonprogressors and progressors begins at an unknown time before detection of Barrett’s esophagus in the clinic. By the time of diagnosis of Barrett’s esophagus, nonprogressors are characterized by an initial expansion of SCA primarily at fragile sites, including FHIT, CDKN2A, and WWOX, and 9p loss or cnLOH that developed before clinical detection, which typically remains relatively stable without generating progressive chromosome instability, diversity, or further selection of SCA over prolonged follow-up (green line) (A). Progressor genomes evolve in this background of low SCA (green line) with an increase in SCA compared with nonprogressors that becomes detectable 48 months before the diagnosis of EA, with only 18q loss detected at a significantly higher frequency than in nonprogressors before this time (blue dashed line). In the 24 to 48 months before EA diagnosis, total SCA increases markedly (yellow line) with selected larger gains on chromosomes 8 and 15q and losses on 5p, 17p, 18, and Y conferring increased risk of progression as well as a background of coselection of large regions of gains and/or losses on different chromosomes that are detected in individual biopsies. This background of coselection and CIN continues to be detected in individual biopsies with SCA <1,500 Mb in the 0- to 24-month window. This is followed by catastrophic genome doublings that result in leaps in SCA to >1,850 Mb within 24 months of EA diagnosis (brown line, B). This model, based upon longitudinal data in humans in vivo, proposes that the rate of SCA accumulation increases in the population of progressors through selection and coselection of large regions of SCA and catastrophic genome doublings as they approach the diagnosis of EA (C).
instability, generation of diversity, selection, coselection, and genome catastrophes, may drive the process of neoplastic progression and be generalizable across other tumor types. In this regard, it is notable that similar sequences of CIN followed by genome doublings have also been inferred based on data from a single point in space and time in advanced cancers of the breast, ovary, lung, and colon (54). A recent study of ovarian cancer reported a 5-year window of opportunity for early detection, which is similar to our results showing increased SCA within 4 years of the diagnosis of early esophageal adenocarcinoma (56).

A number of previous studies have examined somatic chromosome copy number in Barrett’s esophagus and/or esophageal adenocarcinoma samples using SNP arrays (26, 28–30, 48). The majority of these studies examined alterations in esophageal adenocarcinoma samples taken at a single point in space and time. Gu and colleagues contributed substantially to our understanding that biopsies from Barrett’s esophagus in patients who developed esophageal adenocarcinoma had increased chromosomal aberrations (29). Our study builds on this knowledge, but it is unique from previous studies in several aspects: (i) a case-cohort study design including progressors and nonprogressors with patient samples collected longitudinally according to a systematic biopsy protocol (7) and a strong esophageal adenocarcinoma endpoint rather than surrogate markers such as dysplasia; (ii) highly enriched epithelium separated from the underlying stroma, eliminating confounding signals from normal cells; and (iii) high-density (1 million) SNP arrays providing dense coverage across the genome, allowing smaller lesions to be determined more accurately (37). These unique aspects allow comparison of how indolent Barrett’s esophagus and Barrett’s esophagus that progresses to esophageal adenocarcinoma evolve over time and space in humans in vivo.

We have used esophageal adenocarcinoma as the outcome rather than dysplasia for a number of reasons. Formal statistical criteria for evaluating surrogate biomarkers were developed 2 decades ago (17). Valid surrogate markers need to accurately represent the true end point, in this case esophageal adenocarcinoma, and need to be easily and objectively measured. Neither high-grade dysplasia nor any other grade of dysplasia in Barrett’s esophagus has been demonstrated to be a valid surrogate for esophageal adenocarcinoma. In fact, diagnoses of dysplasia, including high-grade dysplasia are not reproducible as reviewed in Odze (38) and they have high misclassification rates resulting in false positive rates for progression to esophageal adenocarcinoma ranging from 42% to 84% (13, 59). Unfortunately, use of irreproducible measures as outcomes has impaired the ability to compare results from different centers; the multiple limitations of using dysplasia as a surrogate marker for esophageal adenocarcinoma risk have been thoroughly reviewed recently (17).

We have included all esophageal adenocarcinoma detected by surveillance in this study (patients who did not have esophageal adenocarcinoma detected at the baseline endoscopy) in contrast to many studies that have excluded a number of esophageal adenocarcinomas from analysis based on an arbitrary designation as “prevalent” if they were detected during variable follow-up ranging from 6 to 12 months (12, 13, 47). The inclusion of all surveillance detected esophageal adenocarcinomas is reasonable because the cancers were endoscopically invisible, detected only by biopsy, and arose in a setting with genome rearrangements that occur suddenly, potentially within a single cell division (54).

The goal of this study was to elucidate somatic chromosomal evolutionary dynamics during progression to esophageal adenocarcinoma compared with nonprogressors, which are the majority of patients seen clinically. Our results revealed previously unsuspected rapid evolutionary dynamics in Barrett’s esophagus before the development of esophageal adenocarcinoma in contrast to prolonged relative genomic stability in nonprogressors at the level of 1 M SNP arrays. We also found some aspects of rapid somatic evolution, including genome doublings, which are shared across a number of other malignancies. A recent whole exome sequencing study of 149 esophageal adenocarcinomas reported that esophageal adenocarcinoma has one of the highest mutation frequencies among cancers, exceeded only by lung and melanoma (32). Yet selected mutations were found in only 26 genes out of more than 8,000 that were mutated and only 4 genes other than TP53 (72%) had mutation frequencies greater than 10% suggesting that it is unlikely that point mutations in specific genes will provide robust discrimination as predictors of progression to esophageal adenocarcinoma. However, the high mutation frequencies they observed raise the possibility that an increased mutation rate develops early in progression to esophageal adenocarcinoma as a consequence of the genotoxic environment (32). Perhaps mutation rate, an evolutionary measure, may be able to differentiate progressors and nonprogressors before the development of CIN, thereby increasing the window of opportunity for early detection of patients with Barrett’s esophagus at high risk of progression to esophageal adenocarcinoma.

Stringent controls on proliferation in multicellular organisms constrain individual cells to a fitness valley on an adaptive landscape from the perspective of their individual proliferative potential (60). Chromosome instability, selection, and coselection of large chromosome regions, and genome doubling provide redundant copies of genes that may facilitate further evolution of gene copy number and can be viewed as large-effect mutations in which phenotypic leaps can be achieved in a few mutational steps allowing cells to gain higher fitness and a selective proliferative advantage (54, 60). We observed coselection of regions from different chromosomes in individual biopsies beginning in the 24- to 48-month time window (Supplementary Fig. S4). This coselection of genomic regions from different chromosomes may result from structural rearrangements that have been reported to occur more frequently
in esophageal adenocarcinoma than colorectal cancer (32), but whole genome sequencing will be required to determine the extent to which such rearrangements contribute to rapid progression. Alternatively, coselection of unlinked SCAs may occur under strong selection in the toxic gastroduodenoesophageal reflux environment of Barrett’s esophagus by affecting phenotypic variation that negatively or positively regulates proliferation (60, 61).

The cancer paradigm has been built around the concept that neoplastic evolution to cancer is a gradual, deterministic process (62). However, evolution of SCA is a dynamic, stochastic process. The concepts of sudden, punctuated evolutionary events that lead to rapid progression to cancer in cross-sectional studies are relatively new (24, 25, 54). Our study is unique in that we used SCA to explore rate of progression to esophageal adenocarcinoma compared with nonprogressing controls. The temporal dynamics of somatic genomic evolution and the windows of opportunity for early detection are poorly defined in Barrett’s esophagus/esophageal adenocarcinoma. Until these windows are better understood, simply identifying additional biomarkers will not decrease esophageal adenocarcinoma mortality given current screening and surveillance approaches that fail to detect rapid evolution to a lethal esophageal adenocarcinoma and selectively detect indolent Barrett’s esophagus.

Overdiagnosis is defined as diagnosis of “disease” that will never cause symptoms or death during a patient’s lifetime (63, 64). Our findings may explain clinical cohort observations that the great majority of patients with Barrett’s esophagus (90–95%) do not progress to esophageal adenocarcinoma, but die of other causes and that those who do progress to esophageal adenocarcinoma while in surveillance can develop rapidly progressive disease without evidence of esophageal adenocarcinoma at the index endoscopy. Our results may also inform population observations that 95% of esophageal adenocarcinomas are detected at advanced stages when patients become symptomatic without a prior diagnosis of Barrett’s esophagus even though the evidence indicates that they arose in Barrett’s esophagus (65). There is abundant evidence of overdiagnosis of Barrett’s esophagus, but there is no evidence for overdiagnosis of indolent esophageal adenocarcinoma in the population because there has been little change in the proportion of patients found with early stage esophageal adenocarcinoma, whereas esophageal adenocarcinoma mortality has continued to increase rapidly despite current screening strategies (1). However, screening may occasionally detect Barrett’s esophagus that rapidly progresses to esophageal adenocarcinoma that was not diagnosed at the index endoscopy. In a retrospective Danish database study of 11,028 patients with Barrett’s esophagus, 66% of new cases of esophageal adenocarcinomas were diagnosed within 1 year of the index endoscopy (12). Similarly, in a large cohort study of 1,099 veterans with Barrett’s esophagus, 56% of esophageal adenocarcinomas were diagnosed within 25 months of the index endoscopy (13). Our study provides a somatic genomic evolutionary explanation for these clinical observations that the majority of esophageal adenocarcinomas are detected within a short time after the diagnosis of Barrett’s esophagus. Our findings of significantly increased SCA and somatic genomic diversity within 4 years of a cancer diagnosis suggests that rapid somatic genomic evolution may underlie underdiagnosis of rapidly progressing life-threatening disease that presents as an advanced malignancy, whereas the majority of Barrett’s esophagus maintains relative somatic chromosomal stability at the level of 1 M SNP arrays over prolonged periods underlying overdiagnosis of nonprogressing Barrett’s esophagus.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Acknowledgments
The authors thank all the research participants who have made this study possible, C. Karlsen, V. Cerera, H. Kiesel, and P. Christopherson for patient care and research biospecimen coordination, and D. Cowan and T. Watson for database support.

Grant Support
X. Li, P. Galipeau, T. Paulson, C. Sanchez, J. Arnaudo, K. Liu, R. Odze, P. Blount, C. Maley, T. Vaughan, and B. Reid. were supported by National Cancer Institute (NCI) P01CA091955. X. Li and B. Reid were also supported by NCI RC1 CA146973. T. Vaughan was also supported by NCI RO1 CA140657 and Research Scholar #117209-RSG-09-163-01-CNE from the American Cancer Society. S. Self and C. Sather were supported by NCI P30 CA015704. C. Sather and B. Reid were also supported by Fred Hutchinson Cancer Research Center Institutional Funds. M. Kuhner was supported by the Department of Genome Sciences, University of Washington, Washington. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 7, 2013; revised October 1, 2013; accepted October 28, 2013; published OnlineFirst November 19, 2013.
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