Review

Genomics, Endoscopy, and Control of Gastroesophageal Cancers: A Perspective

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SUMMARY

Esophageal adenocarcinoma (EA) is remarkably similar to gastric adenocarcinoma CIN subtype. Current enthusiasm for endoscopic control of EA has little impact on mortality. Current strategies need to be revisited given emerging evidence that many cancers develop rapidly by punctuated and catastrophic genome evolution.

In The Cancer Genome Atlas the goals were to define how to treat advanced cancers with targeted therapy. However, the challenges facing cancer interception for early detection and prevention include length bias in which current screening and surveillance approaches frequently miss rapidly progressing cancers that then present at advanced stages in the clinic with symptoms (underdiagnosis). In contrast, many early detection strategies detect benign conditions that may never progress to cancer during a lifetime, and the patient dies of unrelated causes (overdiagnosis). This challenge to cancer interception is believed to be due to the speed at which the neoplasm evolves, called length bias sampling; rapidly progressing cancers are missed by current early detection strategies. In contrast, slowly or non-progressing cancers or their precursors are selectively detected. This has led to the concept of cancer interception, which can be defined as active interception of a biological process that drives cancer development before the patient presents in the clinic with an advanced, symptomatic cancer. The solutions needed to advance strategies for cancer interception require assessing the rate at which the cancer evolves over time and space. This is an essential challenge that needs to be addressed by robust study designs including normal and non-progressing controls when known to be appropriate. (Cell Mol Gastroenterol Hepatol 2017;3:359-366; http://dx.doi.org/10.1016/j.jcmgh.2017.02.005)

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Cancer is a disease of the genome.

Francis Collins

I was trained as a geneticist. The National Cancer Act was passed in 1971 while I was a graduate student in the University of Washington’s Department of Genetics. It was during this period that I first learned the concept of cancer as a disease that develops and progresses by somatic genomic evolution as later proposed eloquently by Dr Peter Nowell in his 1976 Science classic. I became interested in this concept as a graduate student, but it was difficult to identify a research pathway for a basic PhD geneticist to study cancer as an evolutionary process. I therefore changed my plans for a postdoctoral fellowship and instead entered medical school to learn how to study early stages of neoplasia and their relationship to development of cancer. In the medical school “Gut Course” taught by Dr David Saunders, I realized that the advent of modern endoscopy would allow direct access to premalignant lesions such as those in the stomach and esophagus. This concept was reinforced in my gastrointestinal (GI) rotation with Dr Sidney Truelove at Oxford, who taught me to establish cohort studies for long-term follow-up of GI diseases.

In medical school I was taught then existing concepts of cancer, many of which have subsequently been proven to be outdated or even wrong. One prominent example was the concept that cancer develops by gradual linear accumulation of genetic alterations, which was derived from earlier disease models that have been deeply embedded in medical thought for decades. However, gradual linear evolution of cancer has not been proven rigorously, and a significant amount of recent genomic data support the concept that neoplastic evolution is branched, and some steps in neoplastic evolution occur much more rapidly than others. For example, evidence for development of whole genome doublings (WGDs) (near tetraploidy) has only been possible with advances first in cytometric technologies and cytogenetics and more recently in genomic technologies.

With the advent of modern genomic technologies, it has been well-established that cancers evolve from premalignant fields over time and space in tissues of the body, including Barrett’s esophagus (BE). Cancer is more accurately described as a complex, evolutionary process.
than a molecular disease because of its ability to acquire characteristics that allow it to expand, invade surrounding tissues, metastasize to different parts of the body, and eventually kill the patient. It has consistently evaded attempts at control by therapy, early detection, and prevention.

The 25th anniversary of the Funderburg Research Award comes at a time when recent advances in genomic technologies have made it possible for comprehensive studies such as The Cancer Genome Atlas (TCGA) to be completed in a large number of cancers, including those of the stomach and esophagus. These comprehensive studies have provided potential paths forward and insight into the large reservoir of genomic diversity within advanced cancers that could lead to evolution of resistance to cancer therapies, as well as potentially to endoscopic therapies. This effort has provided an atlas of genetic and genomic alterations as well as other measures such as expression and DNA methylation in addition to other characterizations to improve targeted therapy for advanced cancers. The results of TCGA combined with recent advances in immunotherapy have revolutionized approaches to patients who present with advanced malignancies of the upper GI tract.

The 25th Funderburg anniversary also comes at a time when the National Cancer Moonshot Task Force is releasing reports on achievements and strategies. These and other advances may lead to a future in which the longstanding poor outcomes of gastric and esophageal adenocarcinomas (EAs) could radically improve through implementation of these and other strategies to improve targeted therapy for advanced cancers. The results of TCGA combined with recent advances in immunotherapy have revolutionized approaches to patients who present with advanced malignancies of the upper GI tract.

These advances have been a long time coming, and we need to be careful to match our optimistic predictions with reality-based results. We have learned many things since the passage of the National Cancer Act of 1971. Perhaps the most important thing we have learned during these 45 years is that cancer fights back. Therefore, predictions of victory should include plans to overcome evolution of resistance to therapeutic strategies.

Recently, a novel strategy of cancer interception has been proposed to overcome current limitations to early detection and prevention that are imposed by different trajectories of neoplastic evolution. It has been recognized for decades that early detection and prevention strategies miss cancers that evolve so rapidly that they become detectable only after or between screening and surveillance intervals, respectively (Figure 1). Conversely, current strategies will selectively detect non-progressing conditions because they will remain stable for prolonged periods. This concept has been referred to as length bias sampling in the literature, but relatively little progress was made during the pre-cancer genome era (PCGA) because the mechanisms driving "fast" and "slow" or "indolent" tumors were not understood.

The challenges facing cancer interception are different from those involved in deciding treatment for an advanced cancer. In considering a patient with an advanced, symptomatic cancer, the question is how do we treat? In contrast, when we consider cancer interception, we need to decide whether or not to treat and when and how to treat those who need therapy. To do this with the required precision, the trajectory of somatic genome evolution in time and space must be assessed to determine whether a pre-malignancy will progress and to determine the "window of opportunity" during which those patients who will progress can be identified, diagnosed, and treated appropriately when they need therapy. Although many insights can be gained about cancer evolution from "cancer only" study designs, non-progressing controls and temporal data from progressors will be required to determine the window of opportunity for cancer interception studies.

Gastroenterologists currently play critical roles in screening, surveillance, diagnosis, and treatment of gastric and esophageal cancers, but current approaches are far from "precision" medicine in BE. Physicians also face the full spectrum of ways in which cancer evades attempts at control: (1) failure to detect rapidly evolving cancers that kill patients (underdiagnosis), (2) detection of patients with slowly or non-progressing neoplasms who will never die of esophageal or gastric cancer (overdiagnosis), and (3) initial treatment response followed by evolution of resistance to therapy as a result of branched evolution or other mechanisms. For example, there was high hope that endoscopic ablation would be durable, but multiple studies have shown rapid, substantial rates of recurrence ranging from 9% to 33% with radiofrequency ablation. Another study using argon plasma coagulation and multipolar electrocoagulation with a mean follow-up of 6.4 years reported >70% cumulative incidence of relapse of BE. A recent registry follow-up study reported that 100 patients treated with radiofrequency ablation (from a total of 4982) developed EA during follow-up, 9 of whom died of the cancer. The biological bases for recurrence of BE and EA after ablation in some patients are currently unknown.

Inherited mutations that predispose to gastric cancer or to EA offer the greatest window of opportunity for cancer interception and prevention. In some cases, especially those without a family history, the interpretation of the genetic variants with regard to the risk posed to the patient may be unclear, even including germline variants. It is likely that many practitioners will choose to have such variants evaluated by a medical geneticist. The American College of Medical Genetics and Genomics also provides recommendations, but this will likely be a rapidly evolving field in which many gastroenterologists may well seek the opinion of a medical geneticist.

Multiple EA sequencing studies have also reported mutation signatures including 1 signature that has been reported only in gastric and esophageal adenocarcinomas. Mutation signatures are the result of biological processes that produce mutations. Each signature has both DNA damage and DNA repair components. The signature shared by gastric and esophageal adenocarcinomas may be critical to developing prevention strategies for these cancers. TCGA and other data indicate that EA is genomically similar to the chromosome instability (CIN) subtype of gastric adenocarcinoma with high rates of
However, the 3 other gastric adenocarcinoma subtypes, Epstein-Barr, microsatellite unstable, and genomically stable, appear different and might require different strategies.

In a genomic age in which we can see the possibility or even probability of whole genome sequencing of germline and somatic genomes, approaches to these upper GI cancers and their precursors will require a deeper understanding of the genomic bases for the diseases themselves. Some cancers may contain large numbers of well-characterized oncogenic mutations, whereas the role played by other mutations may be poorly understood.44 In this setting,
teams with expertise that includes medical geneticists and genetic counselors in addition to gastroenterologists, surgeons, and oncologists will become increasingly important in diagnosis and management of patients and families. To accomplish these goals to improve patient care, GI faculty, training programs, and continuing education will need to expand their scope to include genetics and genomics of the constitutive and evolving somatic genomes to ensure appropriate decision-making for screening, surveillance, and therapy and to avoid evolution of resistance to therapy.

There has recently been a proposal for a PCGA analogous to TCGA. Much thought should be given to such a proposal before implementation to ensure that it can achieve the goals of cancer interception, which face different challenges than those of TCGA.

Many of the data supporting the PCGA proposal are based on analyses of spatial data in which an order of events is inferred from genotyping and sequencing of "fields" adjacent to or within an advanced cancer. Enrichment of cells of interest in heterogeneous biological samples through microdissection, or flow cytometric sorting can improve the quality of genomic analyses. Genome sequencing and other genetic data are excellent for this type of analysis of evolutionary descent of "clones" derived from a common ancestor through branched evolution. However, the assumption that screening for an early event will improve detection of the more "dangerous" evolving downstream clones before progression to cancer can be foiled if evolution to cancer progresses rapidly in some clones as described by the concept of length bias.

The possibility that neoplasms evolve at different speeds is rarely considered in current proposals for PCGA-like studies, even though failure to consider the variable of time could easily lead to poor outcomes at both the patient and population levels as documented by underdiagnosis and overdiagnosis involving multiple organs. Some conditions may never evolve beyond the first step and the patient dies of unrelated causes, whereas in other patients different clones may progress so rapidly that the patient develops an advanced cancer before it can be detected (Figure 2). Length bias is believed to underlie many of the challenges to early detection and prevention in many organs, including esophagus, prostate, and breast among many others. As a field, we know a lot about the advanced cancer genome, but we know very little about how neoplasms evolve in time, and this is one of the most critical pieces of information that we need to know for successful cancer interception.

BE and EA arise in a toxic, mutagenic environment that includes reflux of acid and bile as well as swallowed tobacco products. A broad range of evidence indicates that Barrett's metaplasia is a protective adaptation to this hostile reflux environment. This evidence includes expression arrays, proteomics, physiology, and molecular studies that consistently report that benign Barrett's metaplasia has a large number of functions that appear beneficial in the toxic, mutagenic environment of reflux disease, but the origin of this metaplastic adaptation remained a mystery for decades. In the past few years, a number of groundbreaking discoveries in humans and model organisms combined with an ancient human pathology literature have converged on the concept that BE is derived from a normal embryonic rest. In 1952, Johns published a pathology study reporting that the normal human embryonic esophagus has a columnar lining that is replaced by squamous epithelium later in development. This concept can be traced back in the human pathology literature into the 1800s. In 2011, Wang et al reported that p63 null mouse embryos develop intestine-like metaplasia with gene expression profiles very similar to human BE. This epithelium was tracked to a columnar embryonic epithelium that is normally replaced by p63 expressing cells. They also reported that a population of these embryonic cells persisted in adult mice and could be detected in humans at the squamocolumnar junction. A transgenic mouse model with esophageal overexpression of interleukin-1β reported evolution of esophagitis, Barrett-like metaplasia and EA provided a valuable model system linking inflammation to neoplastic evolution.

*There are more things in heaven and earth, Horatio, than are dreamt of in your philosophy.*

William Shakespeare

It might be argued that the solution to the challenges of cancer interception would be to undertake ultra-deep sequencing to detect small "dangerous" clones of mutant cells in different tissues. However, sequencing of physiologically normal human eyelids has revealed an unexpected high mutation rate in aged, sun-exposed skin, revealing "a patchwork of thousands of evolving clones with over a quarter of cells carrying cancer-causing mutations while maintaining the physiologic functions of epidermis." Similarly, many early lesions in BE, including somatic chromosome alterations involving CDKN2A, FHIT, and WWOX, occur at equal frequencies in patients who do and do not progress to EA. These examples illustrate that any PCGA designed to improve patient care will need to include non-progressing precursor lesions and normal controls to interpret the spectrum of genomic alterations that are specific to progression that can be used for cancer interception, while minimizing overdiagnosis and overtreatment in patients who will not progress. As one author recently asked, "Does everyone develop covert cancer?" One of the greatest challenges of a PCGA will be to address the question of which PCGA changes require clinical action to save the patient’s life and what changes will have no consequences during the patient’s lifetime.

What type of genomic assays and measures should be performed? At one extreme, some might suggest a relatively simple and cost-contained approach to exome sequencing only of genes found to be mutated at significant frequency in TCGA studies. However, this might not be optimal for detection of complex rearrangements, including WGDs and fusion genes, that confer high risk at later stages of the transition from PCGA to TCGA. Is the mutation rate something that could be monitored in cancer interception? Should we be performing whole genome sequencing in PCGA? There are many ways to assess WGDs that have been reported in many different types of cancers including
Figure 2. Windows of opportunity for cancer interception in BE. The figure shows the temporal course of neoplastic evolution of fragile sites, TP53 loss, CIN, and WGDs in patients with BE who were followed by prospective endoscopic biopsy surveillance to development of EA (A) or did not progress to EA (B). (A) Currently there is a 2- to 4-year window in which to detect TP53 loss (green), CIN (blue), and WGDs (red) for interception of EA arising in BE. The green, blue stair step, and red sudden increase correspond to the tempo proposed by Baca et al.28 for punctuated (CIN in this case) and catastrophic (WGD in this case) genomic evolution, respectively. Other studies predominantly of advanced EAs have reported other abnormalities, including high mutation rates with whole genome sequencing of EA that reported a median of 26,161 mutations across the genome per tumor (range, 18,881–66,225).42 Other studies have also reported SMAD4 mutations, breakage-fusion-bridge cycles (BFB), chromothripsis, kataegis, gene fusions,77 and oncogene amplification, which typically are detected as later events after TP53 loss but have not been evaluated in prospective studies.20,21 BE that does not progress to EA remains relatively stable at the detection level of 1M single nucleotide polymorphism (SNP) arrays (dashed gray line). Some people may inherit a germline mutation that predisposes to BE/EA that could be detected early in life, potentially extending the window of opportunity for EA interception. In summary, multiple studies have reported that TP53 is the only gene that is mutated at high frequency in BE that progresses to EA and can be detected before diagnosis of EA but not in non-progressing BE.22,42,78 Phylogenetic studies of advanced EAs have also shown that TP53 mutations are early events that typically appear in the trunk of the evolutionary tree.24 (B) In contrast, patients who do not progress from BE to EA remain remarkably stable at resolution of 1M SNP arrays for prolonged times. WGD is an abnormal process in which cells double their genome, typically preceded by CIN, followed by doubling from ~2N (diploid) to ~4N (tetraploid) and subsequent evolution of additional chromosome gains, losses, loss of heterozygosity, and chromosome structural alterations resulting in aneuploid cell populations bearing complex karyotypes descended from whole genome doubled cells. This process can be inferred computationally from SNP and copy number array or sequencing data or measured directly by flow or image cytometric detection of abnormally high 4N cell fractions (>6%) and aneuploidy. (B) Patients who did not progress to EA had recurrent small lesions in fragile sites, including the genes FHIT, CDKN2A, and WWOX, detected by 1M SNP arrays. TP53 is the only gene mutation that has been detected in a stage-specific manner for early detection of early EA in BE.22
EA. What is the best way to assess WGDs in a PCGA? In TCGA, WGDs were inferred to occur at an earlier time by computational analyses. However, for cancer interception, we would ideally like to detect WGD in real time as they appear. In the transition of BE to EA, the window of opportunity for inferred detection of WGDs by single nucleotide polymorphism arrays is about 2 years. Flow and image cytometry both detect abnormal increased cell cycle 4N (G2/tetraploid) fractions greater than 6.0% of the cells in a biopsy. These abnormal 4N fractions can then be further evaluated for genomic and expression alterations. Because of the large number of cancer types that have been inferred to undergo WGDs and the evidence that they frequently occur before cancer, how should a PCGA directly assess them at their earliest possible clinical detection when they constitute only about 6%-15% of the cells in a biopsy? This is especially important because there is evidence that WGDs can be substantially reduced in BE by aspirin and other nonsteroidal anti-inflammatory drugs. How does this affect the genome doubling and cytometry gastric cancer literature in which TCGA has reported 4 types, only 1 of which is similar to EA?

In summary, there can be great benefit from a PCGA. However, the different challenges facing cancer interception versus treatment of advanced cancers will likely require different study designs. To develop robust cancer interception strategies, we will need robust assessments of neoplastic evolution including generation of heterogeneity on which natural selection acts. EA develops as a result of somatic genome instability that generates mutations and chromosome abnormalities that lead to expansion of clones with genetic variants, genetic heterogeneity, and progression of these variants to EA. To develop robust cancer intervention strategies, we will need robust measures of these clones as they evolve in space in the esophagus over time. We will also need to recognize that resistance to therapies that logically should work successfully, such as ablation of BE, is alerting us that our current approaches and the thought processes that drive them need to be reassessed, and the vision for the structure of a PCGA needs to be rigorously discussed in public forums to improve success with the final clinical goals.

Bringing advances in genomics and immunotherapy to precision strategies for cancer interception and treatment will require profound shifts in clinical approaches to prevention, early detection, and therapy of advanced upper GI cancers. Whole genome sequencing of both the inherited germline (constitutive) genome and the somatic (gastric and esophageal) neoplastic/metaplastic genomes could greatly improve the efficacy of cancer interception strategies that are based on the trajectory of neoplastic evolution.

Success will depend on forging new paths. We do not need more ways to screen for BE; we need better ways to screen for high-risk BE. One such approach might be to develop non-endoscopic screening methods, such as the Cytosponge (Medtronic, Minneapolis, MN), to detect high-risk BE before development of advanced EAs. The current iteration of Cytosponge would only detect TP53 mutations, and there are no other obvious mutations beyond those affecting the TP53 pathway that would add significant value to the test. One option might be to add other measures with high sensitivity and specificity such as chromosome regions of high risk or other “biomarkers” to the Cytosponge test.

The success of any approach will ultimately rest on the ability to control evolution of the neoplasm and thereby greatly prolong disease-free survival with a very high quality of life for the patient. Current strategies have not been shown to consistently achieve these goals for gastric and esophageal cancers.

Two leading experts have summarized the current state of EA control: “The current strategy can be construed as representing not a ‘war’ on oesophageal adenocarcinoma, but rather a war on Barrett oesophagus. However, for the majority of patients, Barrett oesophagus is a benign condition that usually remains undiagnosed. In fact, the metaplastic epithelium might actually protect against the inflammatory and erosive effects of bile and acid reflux.” Medtronic recalled all lots of the Covidien Cytosponge Cell Collection device after two reports of the device detaching from the removal string during the withdrawal from the patient’s esophagus (FDA Recall Z-2123-2016).

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