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Genetic and Epigenetic Alterations in Barrett's Esophagus and Esophageal Adenocarcinoma

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Abstract

Esophageal adenocarcinoma (EAC) develops from Barrett's esophagus (BE), a condition where the normal squamous epithelia is replaced by specialized intestinal metaplasia in response to chronic gastro-esophageal acid reflux. In a minority of individuals, BE can progress to low- and high-grade dysplasia (LGD and HGD) and eventually to intramucosal and then invasive carcinoma. BE provides researchers with a unique model to characterize the process by which a carcinoma arises from its precursor lesion. Molecular studies of BE have demonstrated that it is not simply a metaplastic tissue, but rather it harbors frequent alterations that are also present in dysplastic BE and in EAC. Both BE and EAC are characterized by loss of heterozygosity (LOH), aneuploidy, specific genetic mutations, and clonal diversity. Epigenetic abnormalities, primary alterations in DNA methylation, are also frequently seen in BE and EAC. Candidate gene and array-based approaches have demonstrated that numerous tumor-suppressor genes exhibit aberrant promoter methylation, and some of these altered genes are associated with the neoplastic progression of BE. It has also been shown that the BE and EAC epigenomes are characterized by hypomethylation of intragenic and non-coding regions. Given the limitations of histopathology for the diagnosis of BE and particularly dysplastic BE, genomic and epigenomic analyses have the potential to improve the precision of risk stratification. Assays to detect molecular alterations that

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are associated with neoplastic progression could one day be used to improve the pathological assessment of BE/EAC and to select high-risk patients for more intensive surveillance.

Keywords

Barrett's esophagus; esophageal adenocarcinoma; cancer genomics; LOH; aneuploidy; genomic instability; DNA methylation

I. INTRODUCTION

Esophageal cancer can be separated into two major histotypes, esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC), and is the eighth most common cancer worldwide (1). The incidence of EAC has been rising more rapidly than any other type of solid cancer in the US for the past several decades, possibly secondary to the increasing prevalence of risk factors such as obesity (2). EAC is a particularly lethal cancer, with five-year survival rates under 20% (3).

EAC develops from Barrett's esophagus (BE), intestinal metaplasia of the lower esophagus which can then progress through low- and high-grade dysplasia (LGD and HGD) to intramucosal carcinoma and then invasive carcinoma (4). Several concurrent histologic and molecular changes have been described for BE and EAC (5–8). The molecular changes observed include structural genomic alterations (amplifications and deletions, translocations), DNA sequence alterations (e.g. missense mutations), and epigenetic modifications, primarily in the form of DNA hypermethylation and hypomethylation of CpG dinucleotides.

In light of the increased risk of EAC in those with BE, individuals diagnosed with BE are advised to undergo periodic endoscopic surveillance with biopsies of the affected segment in order to detect early histologic changes (i.e. the presence of dysplasia) thought to confer risk for EAC development. Yet because the overall risk of progression to EAC is minimal, a challenge when managing individuals with BE is to balance the risks and costs of endoscopic surveillance with the potential benefit of early identification or prevention of cancer. Assays for molecular alterations in BE samples might ultimately complement histological, demographic, and/or endoscopic data and provide a more accurate prediction of an individual's risk for dysplasia or cancer. This chapter will summarize our current understanding of genetic and epigenetic alterations that underpin the development of BE, dysplastic BE, and EAC, with an emphasis on global alterations observed in BE and EAC.

II. Genetic Alterations in Barrett's Esophagus, Barrett's with Dysplasia, and Esophageal Adenocarcinoma

A. Somatic Genomic Alterations in Barrett's Esophagus

The progression of BE to EAC provides a unique system to characterize the process by which a carcinoma emerges from its precursor state. Genomic studies of BE have revealed that it is not simply a metaplastic tissue; it also harbors frequent somatic alterations. The

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analysis of the process of BE progression has been greatly enhanced by dramatic improvements in genomic technologies, including tools to examine genetic mutations as well as larger structural alterations in cancer (and pre-cancer) genomes.

Early studies of BE identified frequent loss of heterozygosity (LOH) at 17p, 5q, 9p, and 13q (9, 10). 17p and 9p harbor the tumor suppressors *TP53* and *CDKN2A*, respectively, and studies have revealed frequent LOH through mutation (*TP53* and *CKN2A*) or promoter methylation (*CDKN2A*). Galipeau et al analyzed a series of esophageal biopsies from patients with BE+HGD without invasive EAC, finding patients commonly develop 9p LOH prior to the onset of 17p LOH (11). 17p LOH was associated with genomic doubling to a 4N state, consistent with the impact of p53 loss upon genomic instability. When multiple biopsies from a single patient and time point were analyzed, 9p LOH was frequently identified in a larger percentage of the overall area of BE. These data contributed to the development of a popular model where *CDKN2A* loss is thought to be an initiating event in BE progression, while *TP53* alterations are later events, associated with neoplastic progression and aneuploidy.

Beyond aneuploidy, BE progression has been associated with increasing clonal diversity (8). Indeed, the presence of genomically distinct clones in the field of BE has been proposed by some researchers, with data suggesting the potential for certain clones to become dominant over time, i.e. a ‘clonal sweep’ (8, 12, 13).

One limitation of studies of populations with BE is that the vast majority of patients with BE do not progress to cancer, making the contribution of specific genomic alterations to the process of carcinogenesis less certain. More recent prospectively established collections have permitted researchers to study differences in structural genomic profiles in BE patients who did or did not progress to cancer. Li et al studied serial BE biopsies using high-density single nucleotide polymorphism (SNP) arrays. They identified chromosomal instability, genome doubling, and an increase in genetic diversity in BE samples taken within 48 months of EAC diagnosis compared to BE samples from non-progressors. Interestingly, while the genomes in non-progressors were relatively stable with fewer copy number changes, 9p (*CDKN2A*) loss was still identified (14). These results were consistent with the model of aneuploidy being associated with neoplastic progression, but were novel in demonstrating that aneuploidy was acquired just prior to the diagnosis of cancer.

The advent of next generation massively-parallel sequencing technologies has enabled systematic studies of the coding mutations in BE. Agrawal et al performed whole exome sequencing on a set of EAC samples, including two cases of EAC with adjacent BE. They were able to identify the majority of mutations found in EAC in the paired BE tissue, confirming that EAC emerges from BE and showing that many coding mutations are already present in BE, including mutations in the tumor suppressor *TP53* (15). Through sequencing of multiple biopsy samples of BE and EAC from the same patient, Streppel et al identified loss of the tumor suppressor *ARID1A* in both BE and EAC. In a larger cohort of patients this group identified loss of *ARID1A* in 4.9%, 14.3%, 16.0%, and 12.2% of BE, BE with LGD, BE with HGD, and EAC, respectively (16). In addition, by immunohistochemical (IHC)

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staining, they identified abnormal nuclear accumulation of P53 in 34.1% of non-dysplastic BE samples.

The most comprehensive large-scale sequencing study in BE samples to date analyzed 26 genes (selected because they are commonly mutated in EAC) in a collection of non-dysplastic BE, BE with HGD, and EAC samples (17). A striking result of this study was that with the exception of *TP53* and *SMAD4*, the other genes did not show differential mutation rates between BE and EAC, even for bona fide tumor suppressors such as *CDKN2A* and *ARID1A*. While only 2.5% of non-dysplastic BE contained a mutation in *TP53*, 70% of cases of HGD and EAC were *TP53* mutant. Non-dysplastic BE samples were chosen because they showed no signs of progression, thus it is notable that they contained tumor suppressor inactivation. As most of these patients likely never progress to cancer, it will be important to determine whether mutations in genes such as *ARID1A* in BE are markers for increased progression risk.

B. Somatic Genomic Alterations in Esophageal Adenocarcinoma

Modern genomics tools are being widely applied to the study of cancers, including EAC. The earliest efforts used genome-wide array platforms for copy-number analysis and found a wide range of copy-number disruptions in EAC. Nancarrow et al identified frequent copy-number alterations including homozygous deletions at putative fragile sites in the genome at genes such as *FHIT* and *WWOX* (18). Goh and colleagues used comparative genomic hybridization (CGH) to confine regions of amplification to targets that included known oncogenes such as *MYC* and *EGFR* (19). Their results also suggested that patients with highly aneuploid tumors have a poorer prognosis. However, other studies have not validated the relationship between aneuploidy and survival (20, 21).

The resolution of array platforms has recently improved, as have the statistical tools with which to analyze copy-number data to identify significantly recurrent alterations (22). Comparisons across tumor types have also shown that copy-number patterns in EAC are strikingly similarity to those in gastric cancers (22, 23). The copy-number study with the largest number of samples to date evaluated 186 EACs in conjunction with a large set of gastric and colorectal cancers (23). A key finding from this group was that the predilection for recurrent genomic amplifications was an important feature distinguishing EAC (and gastric cancer) from lower intestinal tumors. Rates of genomic deletion, by contrast, were not highly divergent between upper and lower gastrointestinal cancers. Statistical analysis demonstrated that amplifications were highly recurrent at the loci of a number of established oncogenes involved with cell signaling (*EGFR*, *ERBB2*, *KRAS*, *MET*, *FGFR2*), the cell cycle (*CCND1*, *CDK6* and *CCNE1*) and transcription factors (*MYC*, *GATA4* and *GATA6*). Alterations in some of these oncogenes, including *CDK6* and *GATA6*, were validated in other studies (24, 25). Many recurrent deletions were at loci of putative fragile site genes, thus their pathologic significance is unclear. As in studies of BE, these data were consistent with a model where aneuploidy and oncogene activation appear to be important precursors for progression to cancer. Similarly, other analyses of these data established that whole-genome doubling is a prominent feature of EAC (18, 19, 26). Newer sequencing technologies that have now been used to characterize EAC have demonstrated relatively

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high somatic mutation rates compared to most other epithelial cancers (15, 17, 26–28). Agarwal and colleagues were the first to publish data focusing on exome sequencing of esophageal cancer. They demonstrated the occurrence of common *TP53* mutations and the absence of Notch family mutations in esophageal squamous cell cancers (15). Dulak et al performed whole exome sequencing on 149 EACs, along with whole genome sequencing of 15 of these EACs (Figure 1) (27). Canonical oncogene mutations in genes such as *KRAS* and *PIK3CA* were uncommon, whereas evidence of oncogene amplification was frequent. By contrast, there were widespread mutations affecting tumor suppressor genes including *TP53* and *CDKN2A* and chromatin-modifying enzymes including *ARID1A*, *SMARCA4* and *PBRM1*. Novel recurrent mutations, including those involving *TLR4* and *ELMO1* were also noted, but their pathologic significance remains unclear.

Utilizing this large-scale sequencing, Dulak and colleagues were also able to evaluate mutation patterns, and found a predilection for A to C transversions at AA dinucleotides. The etiology of these mutations is unknown, but it has been hypothesized to be linked to bile acid exposure and the induction of oxidative DNA damage. This novel mutation signature was also observed in whole genome sequencing of esophageal cancers by other groups (17, 28). The Nones group also performed additional structural analysis of whole genome data, finding that EACs commonly emerge after catastrophic genomic disruptive events termed chromotripsis. These recent genomic studies are consistent with the earlier BE studies in suggesting the significant role of acquisition of aneuploidy in the transition to EAC.

C. Alterations in MicroRNA Expression in Barrett's Esophagus and Esophageal Adenocarcinoma

MicroRNAs are small noncoding RNA molecules which can interact with other RNA molecules, resulting in post-transcriptional regulation of gene expression and gene silencing (29). Although most of the data regarding the role of miRNAs in esophageal cancer pertains to squamous cell carcinoma, there is evidence that *miR-21* and *miR-375* play a functional role in BE and EAC. Several studies have demonstrated that *miR-21* is upregulated in BE and EAC compared to the normal esophagus. Feber et al showed that miRNA expression profiles distinguished normal esophagus from EAC, and that *miR-21* expression was 3–5 fold increased in EAC compared to normal epithelia (30). Meanwhile, another study that utilized microarray-based technology found 34 differentially expressed miRNAs between normal squamous epithelium and BE/EACs, although the miRNA profile did not reliably distinguish BE from EAC (31). In a validation cohort, the five microRNAs chosen for validation with qRT-PCR, including *miR-21*, were successfully able to discriminate normal esophagus from BE/EAC.

There is also evidence that differential expression of miRNAs is associated with the progression of BE to EAC. Revilla-Nuin et al recently identified 23 miRNAs involved in BE progression using miRNA sequencing analysis, finding four miRNAs (*miR-192*, *miR-194*, *miR-196a*, and *miR-196b*) had higher expression in BE patients who progressed to cancer compared to those who did not progress (32).

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III. Epigenetic Alterations in Barrett's Esophagus, Barrett's Esophagus with Dysplasia, and Esophageal Adenocarcinoma

Epigenetics broadly refers to heritable and stable alterations in gene expression that are not mediated by changes in the DNA sequence. Since the discovery of DNA hypomethylation in colorectal cancer in 1982, epigenetic research has revealed an epigenetic landscape consisting of a complex array of epigenetic regulatory mechanisms that control gene expression in both cancer (33, 34) and normal tissue, where it plays a crucial role in embryonic development, imprinting, and tissue differentiation (35). The epigenetic landscape largely impacts the condensation state of the chromatin, determining whether the DNA is accessible to transcription factors and other proteins that control gene transcription (35). The epigenetic mechanisms currently believed to play a role in cancer include: 1) DNA methylation of cytosine bases in CG-rich sequences, called CpG Islands; 2) post-translational modifications of histones, proteins that form the nucleosomes, which regulate packaging of DNA in chromatin; 3) microRNAs and noncoding RNAs; and 4) nucleosome positioning (35). In this review, we will focus on aberrant DNA methylation as it is the most extensively studied epigenetic mechanism in BE and EAC. A number of excellent publications focusing on other classes of epigenetic alterations, such as histone modifications, have recently been written, and the interested reader is directed to those reviews (36–39).

A. DNA Methylation: Overview

DNA methylation refers to the enzymatic addition of a methyl group to the 5-position of the nucleotide cytosine by DNA methyltransferases (DNMT1, DNMT3a, or DNMT3b) to produce 5-methylcytosine, a normal base in DNA (40). Generally, the favored substrate for the DNMTs is the CG dinucleotide sequence, which has been termed CpG. The majority of CpGs are methylated in mammalian cells with unmethylated CpGs being typically present only in regions of DNA called CpG islands, genomic regions 200–500 bases in length with greater than 50% GC content and a ratio of observed-to-expected CpGs greater than 0.6 (41). CpG islands overlap the promoter region of 60–70% of genes and tend to be protected from methylation; however, they can become aberrantly methylated in cancer. CpG methylation can lead to transcriptional inactivation via multiple mechanisms, including directly inhibiting cis-binding elements, including the following transcription factors: AP-2, CREB, E2F, CBF and NF-KB (42–46). Although this aberrant methylation is traditionally correlated with silencing of gene expression, it appears that decreased gene expression is characteristic of only a subset of methylated genes in most cancers (47, 48). Methylation that occurs in CpG sites outside of promoter regions, termed gene body methylation, paradoxically has been correlated with transcriptional activation (49). Moreover, DNA hypomethylation appears to be a prominent epigenetic alteration in BE and EAC and has been associated with increased gene expression (50).

DNA methylation is a normal mechanism in the mammalian genome by which cells regulate gene expression, and gene methylation patterns that are established during embryonic development are maintained in the adult to regulate gene expression. A prominent mechanism by which DNA methylation is thought to regulate gene expression is through

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cooperative interactions with enzymes that regulate the chromatin structure, which can induce a compacted chromatin environment that represses gene expression (48). The interaction between DNA methylation, histone modification and chromatin structure is complex, with abundant crosstalk. DNA methylation can impact chromatin structure, but the converse is also true. Because of the epigenetic crosstalk between DNA methylation and histone modification, aberrant DNA methylation can alter chromatin structure and gene expression, and dysregulation of histones and their modifying proteins may cause aberrant DNA methylation. There is a close association between methylated CpG islands and histones containing repressive post-translational modifications.

Feinberg and colleagues have recently enhanced our understanding of global alterations of DNA methylation in cancer. They have proposed that in addition to CpG islands there are "CpG island shores," areas of less dense CpG dinucleotides within two kilobases upstream of a CpG island, that can also show abnormal methylation in cancer (51). Methylation of CpG island shores is also associated with transcriptional inactivation and splicing alterations and tends to be tissue specific, and has been shown to be altered in colorectal cancer (51, 52). Feinberg and colleagues observed that two thirds of cancer-associated alterations in DNA methylation can be found in large domains, termed 'large organized chromatin lysine modifications' (LOCKs), as well as in smaller regions immediately adjacent to hypermethylated DNA. Their findings suggest a close cooperation between the chromatin state and DNA methylation changes in cancer (53).

B. Epigenetic Alterations in BE and EAC

1. Global alterations in DNA methylation in BE and EAC—Microarray-based technologies have been utilized to interrogate global patterns of DNA methylation in BE and EAC, and to uncover candidate epigenetic drivers of BE progression. One study utilized Illumina HumanMethylation27 BeadChips to interrogate more than 27,000 CpG dinucleotides (54). The authors noted that both BE (N=77) and EAC (N=117) samples were highly methylated compared to normal esophagus (N=94), indicating that epigenetic alterations occurs early in the BE to EAC progression sequence. They also found numerous previously undescribed hypermethylated genes in BE and EAC tissues, including genes encoding ADAM (A Disintegrin And Metalloproteinase) peptidase proteins, cadherins and protocadherins, and potassium voltage-gated channels. Alvi et al also used the HumanMethylation27 BeadChips to compare methylation patterns, focusing on imprinted and X chromosome genes, from 24 BE and 22 EAC samples (55) and validated their findings in retrospective and prospective cohorts to assess the ability of methylated genes to classify individuals as having prevalent BE, dysplastic BE, or EAC. They found four genes (*SLC22A18*, *PIGR*, *GJA12*, and *RIN2*) had the greatest area under curve (AUC=0.988) to distinguish between BE and dysplasia/EAC in their retrospective cohort. In the prospective cohort, this methylated gene panel was able to stratify patients into low, intermediate, or high risk groups based on the number of genes that were methylated.

Kaz and colleagues utilized GoldenGate methylation microarrays (1505 CpGs in 807 genes) to compare methylation of normal squamous (N=30), BE (N=29), BE + HGD (N=8) and EAC (N=30) cases. Distinct global methylation signatures were seen amongst the different

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tissue types, as well as specific genes demonstrating differential methylation between these groups. Within the BE and EAC cases, there were subgroups with distinct methylation signatures (high and low methylation epigenotypes), suggesting that there may be a CpG Island Methylator Phenotype (CIMP) molecular class of BE and EAC (Figures 2 and 3) (56). Further studies are needed to confirm this observation.

In another genome-wide study, massively parallel sequencing was performed in matched BE and EAC tissues and esophageal cell lines to characterize methylation at 1.8 million CpG dinucleotides (57). The authors found that DNA hypomethylation was more frequent than hypermethylated DNA in both BE and EAC cases and that the hypomethylated regions were found in intragenic and non-coding regions. One long noncoding RNA, *AFAP1-AS1*, was highly hypomethylated and overexpressed in BE and EAC tissues and cell lines. When *AFAP1* was silenced using siRNA technologies, esophageal cells exhibited increased apoptosis and reduced proliferation and colon-forming abilities, suggesting a cancer-promoting role for this noncoding RNA in BE and EAC.

2. Specific Epigenetic Alterations in BE and EAC—Aberrant methylation of promoter CpG islands, which leads to gene silencing of a subset of genes, has been shown to occur frequently in BE, dysplastic BE, and EAC. Epigenetic changes involving the promoter regions of several dozen genes have been evaluated using candidate gene approaches based on findings seen in other types of cancers. One of the first tumor suppressor genes shown to be aberrantly methylated in BE was *CDKN2A* (*p16INK4a*), which normally blocks phosphorylation of the Rb protein and inhibits cell cycle progression. *CDKN2A* promoter hypermethylation combined with 9p21 chromosomal loss leads to inactivation of this gene in some cases of EAC or BE with dysplasia (58, 59). CpG island hypermethylation of the *CDKN2A* promoter has been reported in 3–77% of BE cases suggesting that *CDKN2A* methylation is in early event in BE pathogenesis (60–63).

Eads et al evaluated methylation patterns of *APC*, *ESR1*, and *CDH1* in six esophagectomy specimens, which contained both BE and EAC. They analyzed 107 distinct regions of each resected specimen in order to create spatial methylation maps. They found a high incidence of methylation of *ESR1*, *APC* and *CDKN2A* in BE, BE with dysplasia, and EAC in a pattern suggesting simultaneous methylation in large contiguous fields, or clonal expansion of cells that acquired methylation (64). Similar patterns consistent with clonal expansion in BE have been reported in studies that focused on LOH or mutations of *APC, TP53*, and *CDKN2A* (61, 65, 66).

Aberrant methylation of *APC* and *CDH1* in BE and EAC has been evaluated by other groups as well (67, 68). One group found that hypermethylated *APC* was found in 39.5% of BE and 92% of EAC cases, but not in matched normal esophagus. Methylated *APC* could also be detected in the plasma of 25% of EAC patients, and was associated with reduced survival (67). Another group found high levels of methylated *APC* in >95% of BE and EAC, supporting the concept that aberrant methylation of tumor suppressor genes occurs early in the BE→EAC sequence (69).

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Other genes implicated in carcinogenesis have been found to be methylated in BE/EAC, including the STAT-induced STAT inhibitors (SSIs), suppressors of cytokine signaling (*SOCS-1* and *-3*) and *Reprimo (RPRM)* and members of the glutathione S-transferase (GST) and glutathione peroxidase (GPX) family (70–72). Other groups have identified methylation of somatostatin (*SST*), tachykinin-1 (*TAC1*), *NELL1*, *CDH13*, and noted that the incidence of methylation was increased in BE, BE with dysplasia, and EAC versus normal esophageal samples (73–77). In vitro treatment of cultured cells with the demethylating agent 5-aza resulted in increased mRNA expression levels of these hypermethylated genes, substantiating the link between aberrant methylation and gene expression. Additional genes reported to demonstrate aberrant methylation in BE and/or EAC are listed in Table 1. In general, hypermethylation of these genes is detectable in cases of BE without dysplasia, suggesting that many of the epigenetic alterations that occur in EAC are already present in BE.

III. Clinical Implications

A. Risk Stratification and Prognosis for BE

There remains some uncertainty regarding the histological interpretation of BE, BE with LGD, and BE with HGD, and also which patients with BE are at the highest risk for progression to EAC. Given the limitations of standard histopathology, genomic and epigenomic analysis has the potential to aid in risk stratification. Given features such as *TP53* and *SMAD4* mutations, chromosomal instability, and genetic diversity are associated with progressive disease, it is highly feasible that assays for such features could be increasingly used to aid the pathological assessment of disease and to select patients for more careful monitoring and/or ablation of their BE. The results from the Weaver study, showing common tumor suppressor mutations in non-dysplastic BE, demonstrate the need to carefully assess the specificity of genomic markers that might be associated with increased risk of progression (17). Additionally, further development of techniques to optimize sampling of BE tissue is required given the likelihood of clonal diversity within fields of BE. Efforts to procure esophageal tissue samples that are more representative of the entire BE segment will likely increase the proportion of patients with positive genomic findings, making the assessment of the specificity of markers of even greater importance. Emerging non-invasive approaches for epithelial sampling of BE, such as the Cytosponge, could allow cost-effective and safe sampling across the entire field of BE (17).

Maley, Reid and colleagues have conducted numerous studies describing the relationship between clonal diversity and clonal expansions and the risk of BE progression. One prospective study of 268 BE patients evaluated whether clonal expansions during the progression of BE lead to homogenous cell populations or result in clonal diversity (8). The authors found that patients with greater clonal diversity had greater risk of progression to EAC ($p<0.001$). In a follow-up study, this group compared clonal diversity in 79 BE progressors and 169 non-progressors over 20,425 person-months of follow-up, finding that non-progressors had types of chromosomal instability (small localized deletions involving fragile sites and 9p loss/copy neutral LOH) that generated relatively little genetic diversity (14). Individuals that progressed to EAC, meanwhile, developed chromosome instability

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with initial gains and losses, genomic diversity, and selection of somatic chromosomal alterations followed by catastrophic genome doublings. These data suggest that molecular testing to assess risk of progression in BE may need to incorporate assessment of structural genomic alterations and also assessment of multiple foci of BE from individual patients.

B. Prognostic and Predictive Markers for EAC

Current histologic assessment of EAC is relatively uncomplicated, and no histologic subtype has been shown to be associated with any specific genomic alterations (in contrast to gastric adenocarcinoma, where, for example, loss of CDH1 is associated with diffuse-type tumors). Increasingly, however, as more genomic alterations are demonstrated to have utility as prognostic or predictive biomarkers, testing for these changes will become increasingly routine. Currently, the only standard tests performed in EAC measure changes in HER/ERBB2 using a combination of immunohistochemistry (IHC) and fluorescent in-situ hybridization (FISH). The results of these tests are used to guide use of the anti-ERBB2 drug trastuzumab in patients with metastatic disease (78). Clinical trials investigating other candidate gene/genomic targets are ongoing. Over time, it is likely that multiplexed cancer genomic panels will supplant the use of single gene tests. New approaches, including the use of plasma for cancer genome profiling, are under development, which may allow more comprehensive assessment of the heterogeneity of genomic markers in cancer (21).

IV. Conclusions

Barrett's esophagus is a metaplastic tissue that develops in response to chemical injury and is a major risk factor for EAC. The fact that many individuals with BE undergo periodic endoscopy with tissue biopsy means that a valuable source of material to study the molecular changes associated with BE, BE with dysplasia, and EAC is readily available. The molecular changes that have been identified to date include structural genomic alterations, DNA sequence alterations, and epigenetic modifications.

Genomic studies of BE have revealed that it is not simply a metaplastic tissue, but characterized by frequent somatic alterations, including mutations in *TP53* and other genes. BE is also characterized by aneuploidy and activation of oncogenes, both of which appear to be important precursors for progression to cancer. Newer sequencing technologies that have now been used to characterize EAC have demonstrated relatively high somatic mutation rates compared to most other epithelial cancers.

Epigenetic alterations are also frequently found in BE and EAC. Candidate gene approaches as well as genome-wide array-based studies have identified several genes with aberrant promoter DNA methylation in BE and EAC, and in many cases the epigenetic alterations that were found in EAC were also seen in BE.

In general, both genetic and epigenetic abnormalities are seen in BE before the development of dysplasia or EAC. This has important implications if these molecular alterations are to be used as assays to predict the risk of BE progression, since while it may be true that certain tumor suppressor genes are inactivated in many cases of BE, most individuals with BE will not progress to dysplasia or cancer.

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Given the limitations of histopathology, genomic and epigenomic analysis has the potential to improve the precision of risk stratification. Specific gene mutations, chromosomal instability, and genetic diversity are associated with neoplastic progression, and it is foreseeable that assays to detect these features could be used to support the pathological assessment of disease and to select patients for more intensive surveillance.

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Key Points

1. Genetic and epigenetic alterations play a central role in the formation of Barretts esophagus and esophageal adenocarcinoma.
2. Global epigenetic alterations occur early in the Barretts esophagus to esophageal adenocarcinoma sequence.
3. Genomic analysis of esophageal adenocarcinoma and Barretts esophagus has revealed a set of commonly altered genes that are likely drivers of cancer formation in the esophagus.
4. There is considerable genetic and epigenetic heterogeneity in Barretts esophagus and esophageal adenocarcinoma.

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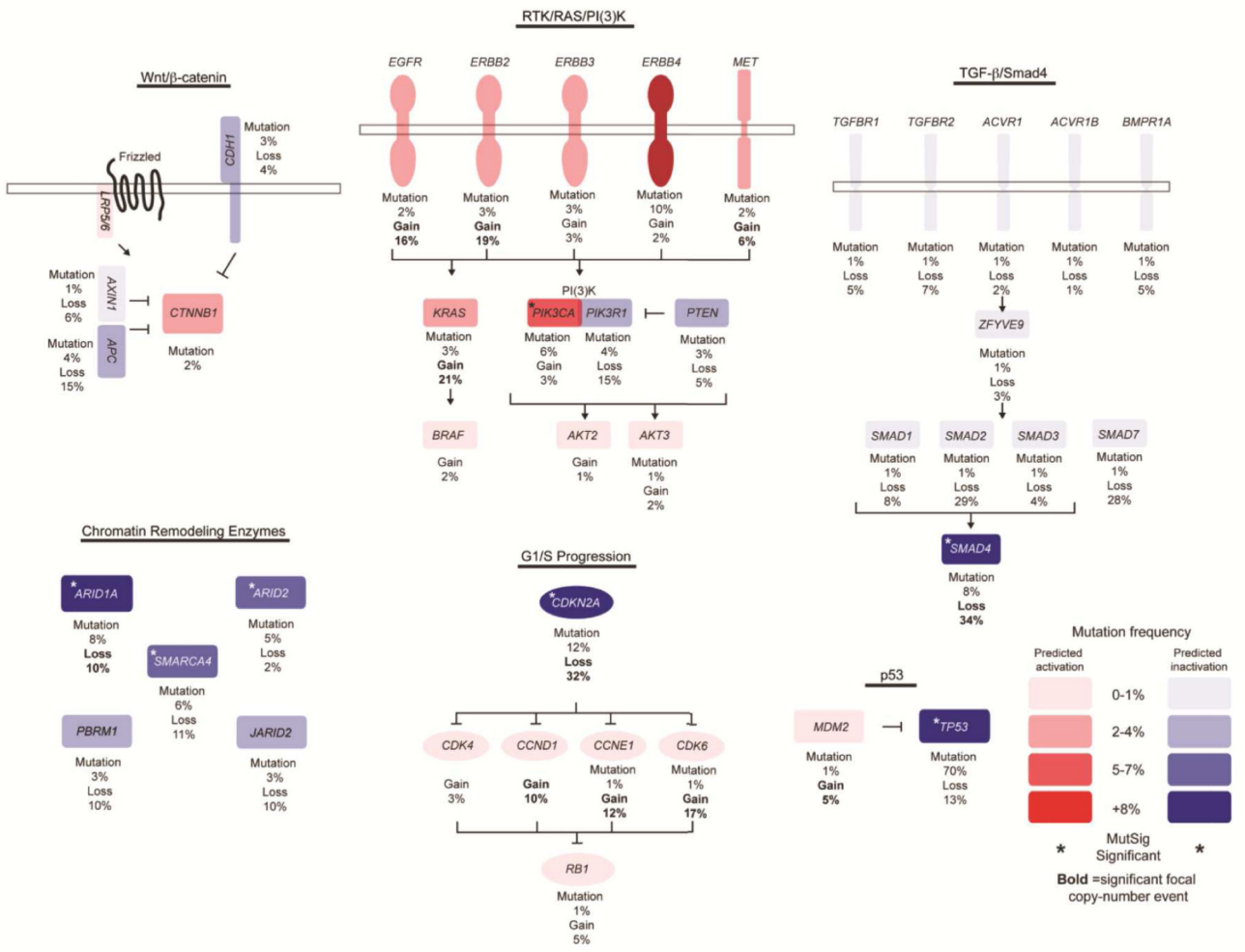

Figure 1.

Genetic alterations and affected pathways in EAC identified by whole exome sequencing. Percentages represent the number of alterations across the cohort. Predicted gain-of-function alterations are represented in red, while loss-of-function alterations are shown in blue. The darker the shade, the more frequently the gene is mutated. From Dulak AM, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, et al. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. Nat Genet. 2013;45(5):478–86; with permission.

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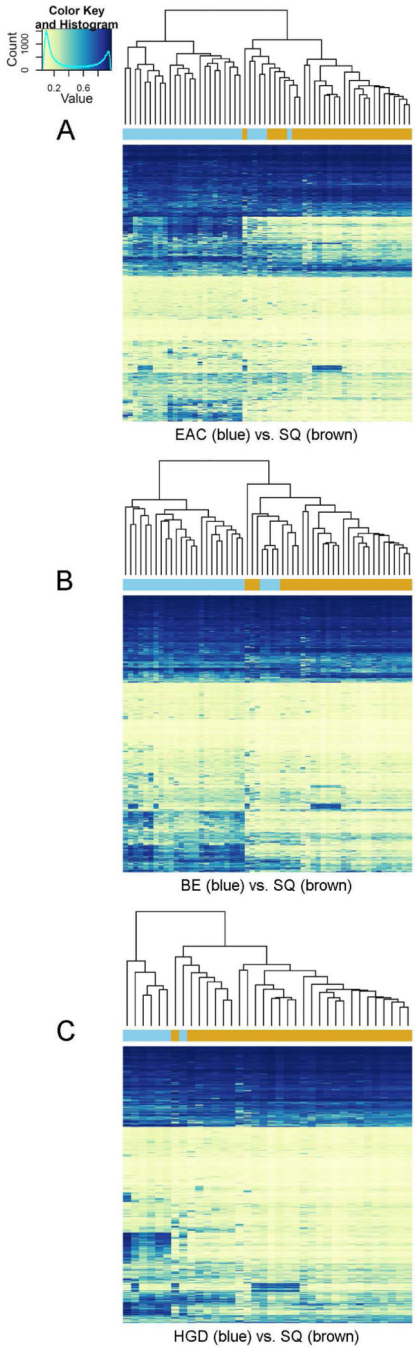

Figure 2.

Dendrograms generated from unsupervised hierarchical clustering analyses revealed distinct methylation profiles based on histological subtype. Each column represents a single sample, and each row an individual CpG site. The darker blue colors correspond to higher β values (i.e., a greater degree of methylation) at particular CpGs. (A) EAC vs. SQ samples. (B) BE vs. SQ samples. (C) HGD vs. SQ samples. From Kaz AM, Wong CJ, Luo Y, Virgin JB, Washington MK, Willis JE, et al. DNA methylation profiling in Barrett's esophagus and

esophageal adenocarcinoma reveals unique methylation signatures and molecular subclasses. Epigenetics. 2011;6(12):1403–12; with permission.

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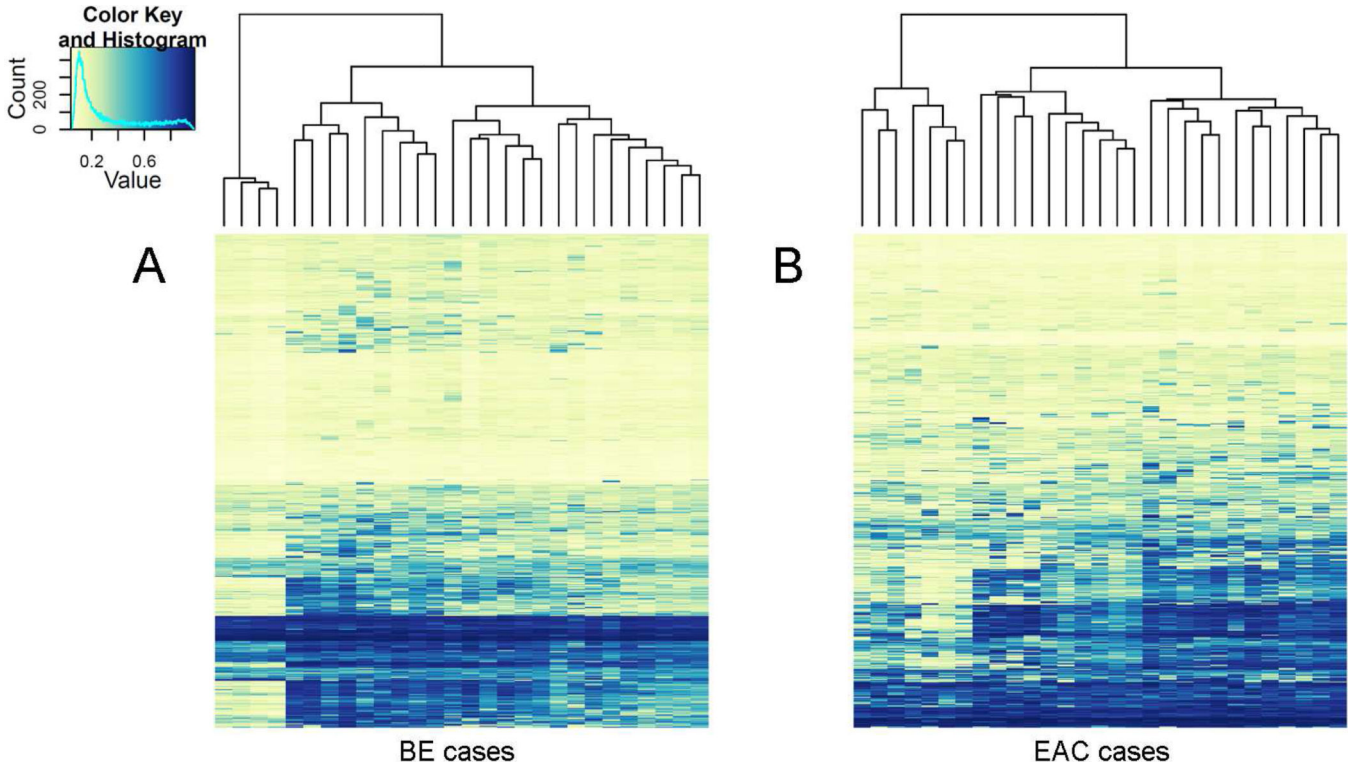

Figure 3.

Dendrograms generated from unsupervised hierarchical clustering analyses within a single histological subtype. Each column represents a single sample and each row an individual CpG site; the darker blue colors correspond to higher β values. When analyzing the BE cases independently (A) or the EAC cases independently (B), we noted two distinct methylation profiles within each of these tissue types: a high and low methylation epigenotype subgroup. From Kaz AM, Wong CJ, Luo Y, Virgin JB, Washington MK, Willis JE, et al. DNA methylation profiling in Barrett's esophagus and esophageal adenocarcinoma reveals unique methylation signatures and molecular subclasses. Epigenetics. 2011;6(12):1403–12; with permission.

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Table 1

Hypermethylated genes in BE, BE with dysplasia, and EAC

Gene	Precursor (M%)	Cancer (M%)	References
CDKN2A	BE (3–77%); IND (60%); LGD (20–56%); HGD (60–75%)	EAC (39–85%)	(58–64, 69, 79–82)
ESR1	BE (69%); LGD (100%); HGD (67%)	EAC (51–100%)	(64, 80)
APC	BE (40–85%); LGD (83%); HGD (66%)	EAC (42–92%; 25% M in plasma)	(64, 67, 69, 79)
CDH1	BE (8%); LGD (0%); HGD (0%)	EAC (0–84%)	(64, 68, 79, 83)
SOCS-1, SOCS-3	BE *SOCS-3* (13%); *SOCS-1* (0%); HGD*SOCS-3* (69%), *SOCS-1* (21%); LGD*SOCS-3* (22%), *SOCS-1* (4%)	EAC *SOCS-3* (74%); *SOCS-1* (42%)	(70)
Reprimo	BE (36%); HGD (64%)	EAC (63%)	(71)
GPX3, GPX7, GSTM2		EAC *GPX3* (62%); *GPX7* (67%), *GSTM2* (69%)	(72)
SST	BE (70%); HGD (71%)	EAC (72%)	(73)
TAC1	BE (56%); any dysplasia (58%)	EAC (61%)	(74)
NELL1	BE (42%); any dysplasia (52%)	EAC (48%)	(75)
AKAP12	BE (39%), any dysplasia (52%)	EAC (52%)	(77)
CDH13	BE (70%); any dysplasia (78%)	EAC (76%)	(76)
DAPK	BE (50%), any dysplasia (53%)	EAC (19–60%)	(80, 84)
VIM	BE (91%); HGD (100%)	EAC (81%)	(85)
SFRP1,2,4,5	BE *SFRP1* (81%), *SFRP2* (89%), *SFRP4* (78%), *SFRP5* (73%)	EAC *SFRP1* (93%), *SFRP2* (83%),*SFRP4* (73%), *SFRP5* (85%)	(86)
EYA4	BE (77%)	EAC (83%)	(87)
p14ARF	BE (7%)	EAC (0–20%)	(63, 79)
MGMT	BE (62%)	EAC (56–64%)	(80, 88)
TIMP-3	BE (72%)	EAC (19–90%)	(80, 89)

*BE=Barrett's esophagus; EAC=esophageal adenocarcinoma; IND=indefinite for dysplasia; LGD=low-grade dysplasia; HGD=high-grade dysplasia; M% = percent of cases demonstrating methylation of given gene

Data from Refs 58–64,67–77,79–89.