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Epigenetic Biomarkers in Esophageal Cancer

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Abstract

The aberrant DNA methylation of tumor suppressor genes is well documented in esophageal cancer, including adenocarcinoma (EAC) and squamous cell carcinoma (ESCC) as well as in Barrett's esophagus (BE), a pre-malignant condition that is associated with chronic acid reflux. BE is a well-recognized risk factor for the development of EAC, and consequently the standard of care is for individuals with BE to be placed in endoscopic surveillance programs aimed at detecting early histologic changes that associate with an increased risk of developing EAC. Yet because the absolute risk of EAC in individuals with BE is minimal, a clinical need in the management of BE is the identification of additional risk markers that will indicate individuals who are at a significant absolute risk of EAC so that they may be subjected to more intensive surveillance. The best currently available risk marker is the degree of dysplasia in endoscopic biopsies from the esophagus; however, this marker is suboptimal for a variety of reasons. To date, there are no molecular biomarkers that have been translated to widespread clinical practice. The search for biomarkers, including hypermethylated genes, for either the diagnosis of BE, EAC, or ESCC or for risk stratification for the development of EAC in those with BE is currently an area of active research. In this review, we summarize the status of identified candidate epigenetic biomarkers for BE, EAC, and ESCC. Most of these aberrantly methylated genes have been described in the context of early detection or diagnostic markers; others might prove useful for estimating prognosis or predicting response to treatment. Finally, special attention will be paid to some of the challenges that must be overcome in order to develop clinically useful esophageal cancer biomarkers.

Keywords

DNA methylation; biomarker; Barrett's esophagus; esophageal adenocarcinoma; esophageal squamous cell carcinoma

1. Introduction

Esophageal cancer, which is the eighth most common cancer worldwide, can be subdivided into two major histologic types: esophageal squamous cell carcinoma (ESCC) and

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esophageal adenocarcinoma (EAC) [1]. The clinical and molecular features of these two cancer types differ in several important ways. Globally, there were an estimated 482,300 new esophageal cancer cases and 406,800 deaths in 2008 [2]. Notably, the incidence rates vary internationally by nearly 16-fold, with the highest rates found in Southern and Eastern Africa and Eastern Asia and lowest rates in Western and Middle Africa and Central America in both males and females. In the highest-risk area, stretching from northern Iran through the central Asian republics to North-Central China, which has been called the “esophageal cancer belt,” 90% of cases are ESCC [2]. Major risk factors for squamous cell carcinomas in these areas are thought to include poor nutritional status, low intake of fruits and vegetables, and drinking beverages at high temperatures. In low-risk areas, which include the US and other developed western countries, smoking and excessive alcohol consumption account for about 90% of the total cases of squamous cell carcinoma of the esophagus. EAC is more common in developed countries for unclear reasons. Risk factors for EAC include smoking, overweight and obesity, and chronic gastroesophageal reflux disease, which is thought to trigger BE. Interestingly, temporal trends in esophageal cancer rates for the two major histological types differ within countries and across countries. The incidence rates for EAC have been increasing in many western countries, possibly secondary to increases in the prevalence of known risk factors such as obesity. In contrast, rates for ESCC have been steadily declining in these same countries because of long-term reductions in tobacco use and alcohol consumption. However, ESCC has been increasing in certain Asian countries such as Taiwan, possibly because of increases in tobacco use and alcohol consumption [2].

Most EAC originates in Barrett's esophagus (BE), a pre-malignant condition where the squamous epithelium of the tubular esophagus is replaced by specialized intestinal-type columnar epithelium [3]. EAC appears to arise via a metaplasia-dysplasia-carcinoma sequence whereby Barrett's metaplasia progresses through low-grade dysplasia, high-grade dysplasia, intramucosal carcinoma, and finally becomes invasive carcinoma [3]. ESCC, meanwhile, is thought to develop from a hyperproliferative epithelium which progresses to low, intermediate and high-grade dysplasia followed by invasive cancer [1]. Although the molecular events that drive these processes are still being sought after, several predictable histologic and concurrent genetic changes have been described for both ESCC and EAC [4–7]. In addition, epigenetic modifications, primarily in the form of DNA hypermethylation of tumor suppressor genes, have been demonstrated to occur frequently in both ESCC and EAC, as well as in the EAC precursor lesion BE [8–11]. A subset of these aberrantly methylated tumor suppressor genes are predicted to play an important role in the pathogenesis of these esophageal cancers. Furthermore, some of these methylated genes might be useful prognostic markers as they appear to precede and thus predict the progression of BE to EAC or dysplasia to ESCC [8].

The search for biomarkers for either the diagnosis of BE, EAC, or ESCC or for risk stratification of EAC in those with BE is currently an area of active research. Because BE is a well-recognized risk factor for the development of EAC, individuals diagnosed with BE are typically enrolled in endoscopic surveillance programs aimed at detecting early histologic changes (i.e. the presence of dysplasia) thought to confer risk for cancer development. Yet the absolute risk of EAC in individuals with BE is minimal (~0.5% or less per year) and 90–95% of individuals with BE will not develop cancer [12–14]. Thus, a challenge in BE is to identify the subset of individuals with the greatest propensity to develop EAC and target them for more intensive surveillance. Molecular alterations, either in the form of large-scale DNA changes, mutations, or methylation might complement or replace histological analysis as more useful biomarkers. Currently, clinicians depend upon the presence or degree of dysplasia to risk stratify individuals with BE as there are no molecular biomarkers that have been translated to widespread clinical practice.

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The purpose of this review is to summarize our current understanding of previously identified candidate epigenetic biomarkers for BE, EAC, and ESCC. Most of these aberrantly methylated genes have been described in the context of early detection or diagnostic markers, while others might prove useful for estimating prognosis or predicting response to treatment. Finally, special attention will be paid to some of the challenges that must be overcome in order to develop clinically useful esophageal cancer biomarkers.

2. Hypermethylated genes in BE and EAC

The tumor suppressor *CDKN2A* (*p16INK4a*), which blocks phosphorylation of the Rb protein and inhibits cell cycle progression, was one of the first genes shown to be aberrantly methylated in BE and EAC. Hypermethylation of this gene promoter combined with loss of heterozygosity (LOH) of 9p21 (which contains the *p16INK4a* locus) leads to *CDKN2A* inactivation in some individuals with EAC or BE with dysplasia [15–16]. In an important study that evaluated the methylation frequency of a 20-gene panel in 104 tissue samples from 51 people, *CDKN2A* was found to be methylated in 15% of BE tissue samples and was unmethylated in normal gastric and esophageal tissues [17]. Methylation of the *CDKN2A* promoter was also found to be associated with other established genetic biomarkers in BE, including 17p (*p53*) LOH and increased aneuploidy/tetraploidy, which together are thought to promote the clonal expansion of BE at high risk of transformation and to drive the process of carcinogenesis [18]. Hypermethylation of *CDKN2A* appears to occur early in the metaplasia-dysplasia-carcinoma sequence, with various studies reporting promoter methylation in 3–77% of BE cases [17–20]. These studies suggest that methylated *CDKN2A* might be a useful marker in a noninvasive assay for the diagnosis of BE.

Eads et al expanded upon the *CDKN2A* data with an evaluation of the methylation status of *APC*, *ESR1*, and *CDH1* in six esophagectomy specimens, which contained both BE and EAC. They performed discrete methylation analyses of numerous regions of each resected sample to create spatial methylation maps comprised of 107 sites per specimen. They found a high incidence of methylation of *ESR1*, *APC* and *CDKN2A* in BE, BE with dysplasia, and EAC in a pattern suggesting clonal expansion of those cells that had acquired methylated alleles of these genes; in contrast, *CDH1* was unmethylated in almost all of the samples [21]. These studies suggest that aberrant methylation of these genes occurs in contiguous fields, possibly indicative of clonal expansion of a hypermethylated cell or group of cells. Similar patterns consistent with clonal expansion in BE have been reported in studies that focused on LOH or mutations of *APC*, *TP53*, and *CDKN2A* [22–23]. Others have also examined the methylation status of *APC* and *CDH1* in BE and EAC [24–25]. Hypermethylated *APC* was found frequently in both EAC and ESCC cases (N=48/52 cases (92%) and N=16/32 cases (50%), respectively) as well as in N=17/34 (39.5%) BE patients, but not in matched normal esophageal tissues. Interestingly, Kawakami et al detected methylated *APC* in the plasma of 25% of EAC patients (N=13/52) and 6.3% ESCC patients (N=2/32). High plasma levels of hypermethylated *APC* were statistically associated with poorer survival [24].

The methylation status of *REPRIMO*, a tumor suppressor gene that regulates p53-mediated cell cycle arrest, was evaluated in 175 endoscopic biopsy specimens and was found to be methylated infrequently in ESCC (13%), and more frequently in BE (36%), BE with high-grade dysplasia (HGD; 64%) and EAC cases (63%) suggesting this might be a useful biomarker for the early detection of esophageal neoplasia [26]. Others have evaluated members of the glutathione S-transferases (GST) and peroxidases (GPX) using a combination of sequencing, real-time PCR, and immunohistochemistry techniques in order to determine whether any were subject to hypermethylation in EAC [27]. This group found frequent hypermethylation of *GPX3* (62%), *GXP7* (67%), and *GSTM2* (69%) (N=75) that was associated with reduced levels of the corresponding mRNA and which was reversible

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following treatment with the DNA methyltransferase 1 (DNMT1) inhibitor 5-aza-2'-deoxycytidine. The suppressors of cytokine signaling (*SOCS-1* and *-3*), which have previously been implicated in liver and head and neck cancers, were evaluated in a collection of normal, metaplastic, and cancerous esophageal tissues [10]. *SOCS-3*, and to a lesser degree *SOCS-1*, was found to be hypermethylated and associated with a subsequent reduction in mRNA transcript levels in BE (*SOCS-3*: N=4/30 (13%), *SOCS-1*: N=0/30 (0%)), BE with low-grade dysplasia (*SOCS-3*: N=6/27 (22%), *SOCS-1*: N=1/27 (4%)), BE with high-grade dysplasia (*SOCS-3*: N=20/29 (69%), *SOCS-1*: N=6/29 (21%)), and EAC cases (*SOCS-3*: N=14/19 (74%), *SOCS-1*: N=8/19 (42%)).

The incidence of DNA methylation of the genes somatostatin (*SST*), tachykinin-1 (*TAC1*), *NELL1*, *CDH13*, and *AKAP12* was evaluated in approximately 260 esophageal tissue specimens in a series of reports [28–32]. In all of these studies, the prevalence of gene methylation was increased in EAC and ESCC DNA as well as in BE and BE with dysplasia as compared to normal esophageal DNA. Experiments in cell lines with the demethylating agent 5-aza-2'-deoxcytidine established the relationship between methylation and reduced mRNA expression levels. The methylation data from the studies referenced above is summarized in Table 1. Additional genes that have previously been reported to demonstrate hypermethylation in BE and/or EAC, including *DAPK*, *SFRP1*, *2*, *4*, and *5*, *EYA4*, *p14ARF*, *MGMT*, and *TIMP-3* are also listed in Table 1 [20; 33–39]. These genes all have potential to be used as diagnostic molecular markers for BE and/or EAC; however, none of them have been subjected to rigorous validation studies.

3. Methylated gene biomarkers for predicting the risk of progression of BE to EAC

Given that Barrett's esophagus only infrequently progresses to high-grade dysplasia or EAC, and that the current clinical guidelines suggest patients with BE undergo regular endoscopic surveillance, a biomarker (or biomarker panel) that could more accurately risk stratify patients with BE would be of great clinical utility. Such a marker could potentially spare the great majority of individuals with a diagnosis of BE from the cost, inconvenience, and minimal risk of regular endoscopy. Being placed in a `low-risk' group might also reduce the feelings of anxiety about developing EAC that have been shown to be associated with a diagnosis of BE [40–41].

Although no genetic or epigenetic biomarkers that estimate the risk of BE progression are in current clinical use, the identification and validation of risk biomarkers is an active area of research. For example, in a retrospective study which compared BE patients who progressed to HGD or EAC to those who did not, hypermethylation of the genes *CDKN2A* (OR 1.74, 95% CI 1.33 – 2.20), *RUNX3* (OR 1.80, 95% CI 1.08 – 2.81), and *HPP1* (OR 1.77, 95% CI 1.06 – 2.81) was associated with an increased risk of progression. Age, BE segment length, and hypermethylation of other genes (*TIMP-3*, *APC*, or *CRBP1*) were not found to be independent risk factors [42]. A follow-up study using these same epigenetic markers was combined with three clinical parameters (gender, BE segment length (SL), and pathologic assessment) in order to generate ROC curves that were able to stratify BE patients into high, intermediate, and low risk for progression to HGD or EAC. This three-tiered risk-stratification method might impact upon the accuracy and efficiency of BE surveillance but has not been adopted into routine clinical use to date [43]. This model was later expanded to include additional genes previously shown to be hypermethylated in BE and/or EAC, most of which have been described in the previous section, to generate an eight-marker risk-of-progression panel. In a retrospective analysis including 145 nonprogressors and 50 progressors, this panel predicted progression with a sensitivity of ~50% when the specificity was set at 0.9 using ROC curves [9].

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In another smaller study, DNA methylation patterns of the genes *APC*, *TIMP-3*, and *TERT* were compared in individuals who progressed to EAC (N=12) to those in individuals who did not progress to EAC (N=16). An increased frequency of methylation of these three genes was found in the progressors versus the nonprogressors (*APC*: 100% vs. 36%; *TIMP-3*: 91% vs. 23%; *TERT*: 92% vs. 17%, respectively), suggesting these genes also might be useful as prognostic molecular markers for estimating the risk of developing EAC [8]. Other reports have also noted that methylated *APC* and *CDKN2A* are associated with an increased risk of BE progression to EAC [44].

4. Hypermethylated genes in ESCC

Although ESCC is not as well characterized as EAC from an epigenetic standpoint, several putative tumor suppressor genes have been shown to be frequently hypermethylated in ESCC. *CDKN2A/p16INK4a*, a tumor suppressor that demonstrates DNA promoter hypermethylation in many BE and EAC cases (as outlined above), also exhibits hypermethylation in ESCC. Hypermethylation of *CDKN2A* is relatively common in ESCC cases, ranging from 40–62%, and is frequently associated with loss of expression and an advanced histological grade of cancer [45–47]. ESCC has been associated with exposure to nitrosamines, which leads to alkyl-related DNA damage that is normally repaired by enzymes such as O(6)-methylguanine DNA methyltransferase (*MGMT*). For this reason, inactivation of *MGMT* by aberrant DNA methylation might favor the progression of esophageal squamous epithelium to ESCC. In fact, methylated *MGMT* has been shown in 33–39% of ESCC cases, and can be associated with a reduction in MGMT protein levels [45; 48].

Furthermore, a microarray analysis of more than 800 genes in a tissue sample set that included multiple ESCC and matched normal cases demonstrated 37 differentially methylated CpG sites, including genes involved in IL-10 anti-inflammatory signaling and cell communication. Methylated *TFF1* was also identified as a potential early marker for ESCC in this analysis [49].

Aberrantly methylated genes have also been detected in the plasma of patients with ESCC. Just as some individuals with EAC have hypermethylated *APC* detectable in their plasma, a minority of ESCC patients (N=2/32; 6.3%) had quantifiable methylated *APC* detected in their plasma [24]. In another study, 23% of patients (N=7/31) who had methylated *CDKN2A* in their ESCC also had this same methylation change detected in DNA isolated from their serum [50].

Numerous other genes have been shown to be hypermethylated in ESCC, and these are listed in Table 2 [26; 28–31; 45; 51–61]. Just as with BE and EAC, these methylated genes have the potential to be used as diagnostic or prognostic molecular markers for ESCC. A major limitation at this time is the lack of robust validation studies to confirm the accuracy of these methylated genes as biomarkers for ESCC so that they can be adopted into clinical practice, if appropriate.

5. Epigenetic biomarkers for prognosis and disease recurrence

Currently, tumor grade, stage, histological type, and residual disease following surgery are the most commonly used clinical parameters to predict prognosis in esophageal cancer. Although these parameters are the best available prognostic markers, they are suboptimal for the accurate prediction of an individual's disease-free and overall survival [1]. In order to improve the accuracy of the determination of an individual's prognosis, recent research has focused on genetic and epigenetic changes that might improve the precision of the assessment of an individual's survival after diagnosis and treatment of esophageal cancer.

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Brock et al examined the methylation status of seven genes in 41 esophagectomy specimens containing EAC with matched normal tissue, and found increased methylation in the genes *APC*, E-cadherin (*CDH1*), *MGMT*, *ER*, *CDKN2A*, *DAPK*, and *TIMP-3*. Individuals with >50% of their gene profile showing aberrant methylation had significantly reduced survival ($p = 0.04$) and earlier tumor recurrence ($p = 0.05$) compared to those individuals with <50% of their genes showing aberrant methylation. A positive methylation status was a better predictor of survival than either age or tumor stage [38]. Other methylated genes that have been associated with a poor prognosis in EAC include methylated *NELL1* and *TAC1* [29–30].

The *APC* gene, which demonstrates frequent methylation in both EAC and ESCC cases, has been associated with reduced survival in ESCC patients following treatment of their disease. In a cohort of ESCC patients (N=45), 44.4% had hypermethylated *APC* detected in their cancers, and this group showed reduced two-year survival rates as compared to those with unmethylated *APC* present in the cancers [62]. Methylated *FHIT* has also been associated with a poor outcome. In a study of ESCC patients (N=257), 33% had methylated *FHIT* present in their cancers, and these cases were associated with a greater rate of disease recurrence after esophagectomy (HR = 5.81 (CI = 1.15–14.07) versus controls), as well as reduced survival after recurrence (HR = 2.31 (CI = 1.18–7.92) versus controls) [63]. Another study focused on the carboxyl-terminal ubiquitin hydrolase family member *PGP9.5* in a series of primary ESCC tumors (N=50). Patients with the highest *PGP9.5* methylation levels had poorer five-year survival rates ($p = 0.01$) and also an increased incidence of lymph nodes metastases ($p = 0.03$) versus those with lower methylation values [61]. Additionally, the tumor suppressor gene *TSLC1* is frequently methylated in ESCC cases, and methylated *TLSC1* has been associated with loss of TSLC1 mRNA expression and aggressive tumor behavior [60].

Other studies have evaluated the utility of epigenetic biomarkers for estimating the risk of esophageal cancer recurrence after treatment. Methylation of the Wnt antagonists *SFRP1*, *DKK3*, and *RUNX3* in DNA isolated from the plasma of ESCC patients has been associated with an increased risk of recurrent disease [64]. Patients (N=81) with hypermethylation of two out of these three markers were shown to have an elevated risk or recurrence, with an OR of 15.69 (95% CI = 2.97–83) compared to those with no methylated genes detected in their plasma. In another report, recurrence of Stage I ESCC was associated with *CDH1* methylation (OR = 5.26, 95% CI = 1.48–18.67) and the risk of recurrence was elevated in those with methylated *WIF1* detected in their ESCCs (HR = 13.17, 95% CI = 2.46–70.41). For Stage II cancers, methylated *ITGA4* (the gene for integrin-alpha4) was associated with an increased risk of cancer recurrence (OR = 3.03, 95% CI = 1.09–8.37) and reduced recurrence-free survival (HR = 2.12, 95% CI=1.13–3.98) compared to those without methylated *ITGA4* [65]. In another study of patients with either ESCC (N=50), esophageal dysplasia (N=50), or no disease (N=50), the promoter methylation status of nine cell-cycle associated genes was examined by methylation specific PCR [66]. The frequency of promoter methylation was 52% for $p14^{ARF}$, 44% for *p15*, 50% for *CDKN2A*, 56% for *CDKN1B/p21*, 38% for $p27^{KIP1}$, 8% for *TP53*, 42% for *p57*, 36% for *p73*, and 44% for *RB1* in the ESCCs. In this study the tumors were defined as having a CpG island methylator phenotype (CIMP) if ≥5/9 genes were methylated. The authors detected CIMP in 54% (N=27/50) of ESCC and 8% (N=4/50) of dysplastic tissues. They did not detect CIMP in any normal epithelial tissues. A significant difference between CIMP status and TNM stage and metastasis was found in the ESCCs. Furthermore, patients with ESCC with CIMP were found to have a worse four-year survival rate compared to patients with non-CIMP ESCC.

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6. Biomarkers to predict treatment response

Since most patients with esophageal cancer have a poor clinical outcome with surgical treatment alone, neoadjuvant chemoradiotherapy is recommended for many individuals. Molecular markers that might predict response to chemo or radiotherapy would be highly valuable to clinicians planning treatment as they would allow customization of the treatment regimens to maximize benefit while limiting the toxicity associated with these therapies. Methylated genes are likely to alter a tumor's response to treatment as many of these genes are known to regulate DNA damage repair (e.g. *MGMT*, *MLH1*, *BLM*), proliferation (e.g. *CDKN2A*, $p14^{ARF}$) and apoptosis (e.g. *PTEN*). Methylation would be predicted to affect treatment response, since inactivation of these particular genes might result in certain tumors demonstrating either an enhanced or attenuated response to chemoradiotherapy [67].

When chemoradiation responders (N=13) and non-responders (N=22) with esophageal cancer were compared in one study, the number of methylated genes was found to be lower in responders (1.4 versus 2.4 genes per patient when the genes CDKN2A, REPRIMO, p57, p73, RUNX3, CHFR, MGMT, TIMP-3, and *HPP1* were analyzed) [11]. With respect to individual genes, in one study, methylated *REPRIMO* was detected at significantly lower levels (and less frequently) in chemoradiotherapy responders versus nonresponders [26].

7. Genome-wide methylation studies in BE and EAC

Genome-wide studies of methylation patterns in BE and EAC have the potential to shed light on differential patterns of DNA methylation among various esophageal tissue types, to define the molecular events involved in the progression of BE, and to uncover numerous additional epigenetic biomarkers. One such study utilized methylated CpG island amplification (MCA) and Agilent 244K Human CpG island microarrays to compare BE patients who progressed to cancer (N = 5) to BE patients that did not progress (N = 4) [68]. In this study, BE progressors were more likely to demonstrate *hypomethylation* of growth-promoting genes (as opposed to hypermethylation of tumor suppressor genes) compared to non-progressors, including genes involved in insulin signaling pathways. Additionally, they found certain genes became demethylated early during the process of progression whereas others became demethylated closer to the point of progression to high-grade dysplasia or cancer.

Another study incorporated a combination of microarray-based assays that assessed genome-wide DNA methylation, gene expression, and chromosomal DNA alterations (array comparative genomic hybridization (CGH)) in an attempt to define the molecular events underlying the progression of BE to esophageal adenocarcinoma [69]. The results suggested that the major change to occur during progression was loss of methylation, which occurred relatively early in the process of carcinogenesis. Global hypomethylation cooperated with gene amplification, leading to upregulation of CXCL1, CXCL3, GATA6, and DMBT1, which might be functionally important cancer-related proteins and which have the potential to be biomarkers used to screen patients with BE for neoplastic progression.

8. Obstacles to the discovery of useful biomarkers for BE and esophageal cancer

The clinical application of methylated DNA biomarkers for both diagnosis and prognosis of BE and esophageal cancer is hindered by the lack of adequate validation clinical trials (Phase 2–3 biomarker studies) [70]. A thorough review by Prasad et al summarizes many of these issues which are not unique to the field of esophageal cancer but are problematic for cancer biomarkers in general [71–72]. Most of the epigenetic biomarkers described in the

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current review are Phase 1–3 biomarkers with only `any *p16* lesion' (which includes hypermethylation, LOH, and sequencing of *p16INK4a*) being a Phase 4 biomarker as defined by the Early Detection Research Network (EDRN) [70; 73]. The primary barrier to developing clinically useful biomarkers is the lack of suitably large prospective clinical trials, which are hindered by the lack of sizeable esophageal tissue repositories that include complete clinical annotation. The design and implementation of large-scale trials will likely require multi-institutional cooperation and significant funding in order to generate the cohorts needed to validate the promising biomarkers that have been identified to date [42; 71; 74–75].

One approach that can be used as an intermediate step between Phase 1 discovery studies and prospective cohort studies is the evaluation of retrospectively collected patient populations. The use of formalin-fixed, paraffin-embedded (FFPE) esophageal specimens from retrospective, clinically-annotated tissue collections for molecular studies provides one way to partially overcome the limitation of tissue availability. The DNA isolated from FFPE samples is generally stable for many years and can typically be used for genome-wide, microarray analyses. Although studies utilizing esophageal DNA are quite limited at this time, our group recently used the Illumina GoldenGate platform to show that normal squamous esophagus, BE, and EAC cases have unique `methylation signatures' [76]. Several genes demonstrated differential methylation between the histological groups, and there was evidence of `high-methylator' and `lowmethylator' subtypes within the BE and EAC cases, similar to the CpG island methylator phenotype (CIMP) that has been described with many other cancer types [77–78]. Although additional genome-wide and methylome-wide microarray analyses with large numbers of clinically-annotated esophageal cases are necessary for the validation and development of biomarkers for diagnosis and progression, the assessment of these retrospectively collected tissue sets will allow further analysis of promising biomarkers while the prospective patient collections are in progress.

9. Conclusions

In summary, there are a myriad of published studies of aberrantly methylated genes in BE, EAC, and ESCC in the literature to date (N=311, PubMed search terms “DNA methylation” and “esophageal cancer”). Although many of these studies involve the analysis of relatively few patients and are generally not prospective in nature, hypermethylated tumor suppressor genes appear to be associated with Barrett's esophagus and esophageal cancer and thus show considerable potential to be used as diagnostic biomarkers. Additionally, in some cases, the hypermethylation of specific genes has been shown to be associated with clinical outcomes, including disease prognosis or response to treatment, which demonstrates the potential of methylated genes to also serve as prognostic or predictive biomarkers. More recently, genome-wide, microarray-based approaches have begun to uncover additional differences in the methylome between the normal esophagus, esophageal precursor lesions, and esophageal cancer. Further evaluation of the differentially methylated genes between these groups, in the form of relatively large, prospective clinical trials, is needed in order to develop clinically useful biomarkers for the management of individuals with esophageal cancer or BE.

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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%)	[15–16; 18–21; 36; 38–39; 79]
ESR1	BE (69%); LGD (100%); HGD (67%)	EAC (51–100%)	[21; 38]
APC	BE (40–85%); LGD (83%); HGD (66%)	EAC (42–92%; 25% M in plasma [24])	[21; 24; 36; 38]
CDH1	BE (8%); LGD (0%); HGD (0%)	EAC (0–84%)	[21; 38; 80]
REPRIMO	BE (36%); HGD (64%)	EAC (63%)	[26]
GPX3, GPX7, GSTM2		EAC *GPX3* (62%); *GPX7* (67%), *GSTM2* (69%)	[27]
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%)	[10]
SST	BE (70%); HGD (71%)	EAC (72%)	[28]
TAC1	BE (56%); any dysplasia (58%)	EAC (61%)	[29]
NELL1	BE (42%); any dysplasia (52%)	EAC (48%)	[30]
AKAP12	BE (39%), any dysplasia (52%)	EAC (52%)	[32]
CDH13	BE (70%); any dysplasia (78%)	EAC (76%)	[31]
DAPK	BE (50%), any dysplasia (53%)	EAC (19–60%)	[33; 38]
SFRP1,2,4,5	BE *SFRP1* (81%), *SFRP2* (89%), *SFRP4* (78%), *SFRP5* (73%)	EAC *SFRP1* (93%), *SFRP2* (83%),*SFRP4* (73%), *SFRP5* (85%)	[34]
EYA4	BE (77%)	EAC (83%)	[35]
p14ARF	BE (7%)	EAC (0–20%)	[20; 36]
MGMT	BE (62%)	EAC (56–64%)	[37–38]
TIMP-3	BE (72%)	EAC (19–90%)	[38–39]

*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

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Table 2

Hypermethylated genes in ESCC

Gene	Precursor (M%)	Cancer (M%)	References
CDKN2A	ED1 (31%); ED2 (42%); ED3 (33%)	ESCC (40–62%; 23% M in serum [50])	[45–47; 50–51; 81]
MGMT	ED1 (23%); ED2 (17%), ED3 (11%)	ESCC (33–39%)	[45; 48]
APC	ED1 (3%); ED2 (0%); ED3 (0%)	ESCC (50%; 6.3% M in plasma)	[24]
p14ARF		ESCC (15%)	[51]
p15INK4b		ESCC (12%)	[51]
DAB2		ESCC (20%)	[52]
HIN-1	ED1 (31%); ED2 (33%); ED3 (44%)	ESCC (50%)	[53]
MLH1	ED1 (8%); ED2 (17%); ED3 (33%)	ESCC (23%)	[45]
RAR β 2	ED1 (13%); ED2 (33%); ED3 (44%)	ESCC (36–70%)	[45; 58–59]
CDH1	ED1 (10%); ED2 (17%); ED3 (33%)	ESCC (34%)	[45]
DAPK	ED1 (28%); ED2 (25%); ED3 (11%)	ESCC (26%)	[45]
ECRG4		ESCC (60%)	[54]
FHIT	ED1 and ED2 combined (78%)	ESCC (45–69%)	[55; 59]
GNG7		ESCC (41%)	[56]
TPEF		ESCC (54%)	[57]
VHL		ESCC (13%)	[59]
RASSF1A		ESCC (51%)	[59]
TSLC1		ESCC (50%)	[60]
PGP9.5		ESCC (42%)	[61]
REPRIMO		ESCC (13%)	[26]
SST		ESCC (54%)	[28]
CDH13		ESCC (19%)	[31]
TAC1		ESCC (50%)	[29]
NELL1		ESCC (12%)	[30]

*ESCC=esophageal squamous cell carcinoma; ED1=low-grade dysplasia; ED2=intermediate-grade dysplasia; ED3=high-grade dysplasia; M% = percent of cases demonstrating methylation of given gene