Am J Cancer Res 2021;11(11):5644-5658
www.ajcr.us /ISSN:2156-6976/ajcr0139013

Review Article

DNA methylation markers in esophageal cancer: an emerging tool for cancer surveillance and treatment

He Wang[1*], Samuel M DeFina[1*], Manisha Bajpai[2], Qin Yan[1], Lei Yang[1], Zhongren Zhou[3]

[1]Department of Pathology, Yale School of Medicine, Yale University, New Haven, Connecticut, United States; [2]Department of Medicine-Gastroenterology and Hepatology, Rutgers-Robert Wood Johnson Medical School, Rutgers The State University of New Jersey, New Brunswick, NJ, United States; [3]Department of Pathology & Laboratory Medicine, Rutgers-Robert Wood Johnson Medical School, Rutgers The State University of New Jersey, New Brunswick, NJ, United States. [*]Equal contributors.

Received September 9, 2021; Accepted September 30, 2021; Epub November 15, 2021; Published November 30, 2021

Abstract: Esophageal carcinoma (EC) is one of the most pervasive cancers in the world, with upwards of 500,000 new diagnoses, annually. Despite its prominence, advancements in the detection and treatment of EC have been marginal over the past 30 years and the survival rate continues to stay below 20%. This is due to the uncommonly heterogeneous presentation of EC which presents unprecedented challenges in improving patient survival and quality of care. However, distinct epigenetic alterations to the DNA methylome may provide an avenue to drastically improve the detection and treatment of EC. Specifically, the creation of novel biomarker panels that consist of EC-specific methylation markers have shown promise as a potential alternative to the more invasive, contemporary diagnostic methods. Additionally, growing insight into the biological and clinical properties of EC-specific methylation patterns have opened a window of opportunity for enhanced treatment; of growing interest is the application of "DNMT inhibitors" - a class of drugs which inhibit excessive methylation and have been shown to re-sensitize chemoresistant tumors. Here we provide a comprehensive review of the current advancements in EC DNA methylation to underscore a potential approach to its detection and treatment.

Keywords: Esophageal carcinoma, DNA methylation, DNMT inhibitors, cancer precursor lesion

Introduction

Every year, over 500,000 people worldwide are diagnosed with esophageal carcinoma (EC), with approximately the same number of EC-related deaths. The two major subtypes of EC are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). While ESCC, commonly seen in Africa, Asia and South America, is the predominant form of EC, the number of EAC cases has increased 600-800% in developed, western countries over the past 30 years. Due to this increase, EC has become the 6th most common cause of cancer-related deaths in the United States [1, 2]. Both ESCC and EAC are markedly prevalent in men over women, with men accounting for 70% of all EC cases worldwide [3]. In contrast, obesity and gastroesophageal reflux disease (GERD) has been strongly linked to an increased risk for EAC, while smoking and alcohol consumption pose an increased risk for ESCC [4].

Currently, given the high mortality rate of EC and the inefficacy of existing treatments, prevention and early detection continue to yield the best chances of survival. The defining precursors for EAC and ESCC are Barrett's Esophagus (BE) and squamous dysplasia, respectively, with BE representing a 50 to 100-fold increased risk for carcinogenesis [5]. Moreover, these pre-malignant lesions provide an opportunity for the early detection and treatment of ECs, given their locations. Both BE and squamous dysplasia occupy the same regions as their associated malignancies: BE is found to be at the gastroesophageal junction (GEJ) and in the distal third of the esophagus, while squamous dysplasia is found in the proximal two-thirds of the esophagus [3, 6]. At present, the gold standard for EC screening is endoscopy, with biopsy for iodine staining [1]; however, while screening for these pre-malignant lesions offer some forewarning of subsequent EC, BE alone is not an absolute indicator of malignan-

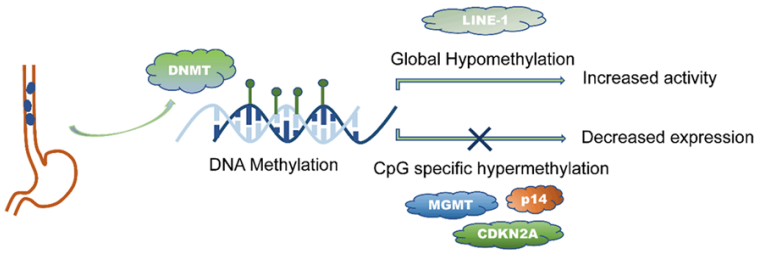

Figure 1. Aberrant DNA methylation in carcinogenesis. DNA methylation, catalyzed by DNMT family enzymes, alter gene function or activity in esophageal cancer. DNA methylation, mainly classified into hypermethylation and hypomethylation, in esophageal cancer alter different gene function or activity. After the interaction of DNMT and DNA binding sites, global hypomethylation causes the enhanced activity of the genes, such as LINE-1, and CpG-specific hypermethylation is correlated to gene expression reduction, such as MGMT and CDKN2A.

cy. Moreover, such invasive and financially burdensome procedures call into question the feasibility of widespread endoscopic screening for EC. Specifically, given the large proportion of ESCC patients residing in impoverished parts of the world, labor-intensive and costly screening methods will only marginally help reduce the mortality rate of EC. For this reason, EC typically remains undiagnosed until advanced stage disease, when the overall survival is typically ≤1 year [1]. Despite significant advancements in medicine and cancer biology over the past several decades, treatment outcomes for EC have only seen marginal improvements, keeping the 5-year survival rate at 15-20% [1].

Despite our understanding of pre-existing conditions which pose increased risks for EC carcinogenesis, the absence of robust molecular markers have largely contributed to the marginal advancements in EC treatments. EC's continuously low survival rate can also be attributed to its exceptionally heterogenous presentation. Thousands of genomic aberrations have been linked to EC [7, 8]. In fact, the extent of EC's heterogeneity is not only seen among different patients, but also, differing genomic alterations have been reported to be present in different sections of the same tumor [9-11]. This degree of heterogeneity has remained a foremost impediment for researchers who try to stratify risk levels associated with disease progression and identify reliable prognostic indicators. Therefore, the establishment of non-interventional, economically feasible screening methods in conjunction with dependable biomarkers are of paramount importance in mitigating EC's lethality.

DNA methylation

Epigenetic markers on DNA are heritable modifications that alter gene function or activity, yet do not change the underlying DNA sequence. One prominent marker in EC is DNA methylation - the process by which Cytosine residues are methylated at the C5 position, therein becoming 5-methylcytosine. DNA methylation is essential for myriad functions, including mammalian development, and is catalyzed by several different proteins, termed epigenetic writers [12], in the DNA methyltransferase (DNMT) family of enzymes. In general, DNMT3a/b are responsible for de novo methylation, while DNMT1 maintains existing DNA methylation by copying methylation patterns onto newly synthesized strands during DNA replication [13]. While DNA methylation is paramount in modulating gene expression across a wide variety of cells, its dysregulation has been linked to carcinogenesis, and is classified into two categories: hypermethylation and hypomethylation. Hypermethylation is associated with gene repression, while hypomethylation is correlated to increased gene expression [14]. DNA methylation changes alter the recruitment of epigenetic regulators and transcription factors to their binding sites [15]. In EC, the two predominant forms of dysregulated methylation patterns are global hypomethylation, and CpG-specific hypermethylation [16, 17] (**Figure 1**).

Over the past several decades, multiple studies have shown that global hypomethylation plays a significant role in carcinogenesis, through several Mechanisms [18-21] (**Figure 1**). Namely, the hypomethylation of parasitic, repetitive DNA sequences, such as retrotransposons, or of proto-oncogenes has pronounced deleterious effects on chromosomal stability and cellular function [22]. Further, excessive hypomethylation of centromeric and pericentromeric satellite sequences is commonly seen in a variety of tumors, and its consequential effects on chromosomal stability has been suggested to lead to aneuploidy [23]. Following its established significance in carcinogenesis, meta-analyses of global DNA hypomethylation

in various cancers have suggested a link between the extent of hypomethylation and cancer stage [22, 24].

In contrast to its activating counterpart, extensive DNA hypermethylation of promoter-region CpG islands has been reported to largely facilitate tumorigenesis by repressing tumor suppressor genes [25]. Numerous studies have since shown the degree to which hypermethylation promotes tumorigenesis. One example of this was shown in the promoter region of the cyclin-dependent kinase (CDK) inhibitor gene, $p16^{INK4A}$, whereby hypermethylation is observed to occur in the pre-cancerous stages of tumorigenesis [26-29]. Another mechanism by which DNA hypermethylation results in aberrant gene expression is through C>T point mutations, via spontaneous deamination of 5-methylcytosine which ultimately is replaced by thymine residues, if not repaired [30]. Such reactivating hypermethylation mutagenesis patterns are often seen in the promoter region of Telomerase Reverse Transcriptase (TERT) complex, which is found in approximately 90% of human cancers, including gastric, pancreatic, and cervical cancers [31-38]. Therefore, given the extent to which aberrant methylation patterns - both of hypo and hypermethylation - influence the onset of carcinogenesis, developing better methods for monitoring alterations to the methylome will play a pivotal role in preventing cancer progression.

Screening for esophageal carcinoma

Current techniques used for the screening of EC are similar and not sufficient for the diagnosis of precancerous lesions. One example being traditional white-light endoscopy (WLE), which is routinely used for the detection of invasive esophageal carcinomas, and even low-grade BE dysplasia. However, WLE is not capable of detecting esophageal squamous dysplasia [39, 40]. A common and inexpensive alternative to WLE is chromoendoscopy, which is sensitive enough to detect precursor lesions, but has insufficient and inconsistent specificity (37%-82%) for squamous dysplasia [41-44]. Other conventional endoscopic methods include transnasal endoscopy, microendoscopy, and endocytoscopy - all of which consist of flexible probes surveying the esophagus (**Table 1**).

To detect aberrant methylation in patients with EC, typically one of the aforementioned endoscopic methods would be used for tissue biopsy, though newer, non-invasive methods such as the Cytosponge or Esophacap are being used to collect cells from the esophageal mucosa. Subsequently, a variety of assays, generally beginning with bisulfite conversion, could be performed to detect methylation [45]. Bisulfite conversion works by deaminating unmethylated cytosine residues to uracil while leaving methylate cytosine residues alone, therein highlighting the presence and location of DNA methylation [46, 47]. Following bisulfite conversion, a number of different assays can be performed, including sanger or pyrosequencing [48]. Other popular methods of DNA methylation detection include array-based platforms and Methyl-cytosine based immunoprecipitation (IP) followed by sequencing [49, 50] (**Figure 2**).

Potential applications of DNA methylation markers in EC

The importance of DNA methylation in cancer was first highlighted in 1983 when significant hypomethylation was observed in cancer cells, in contrast to the surrounding healthy cells [51]. Since then, deleterious epigenetic modifications in ECs have been investigated for a variety of reasons. At present, limitations in our understanding of EC biology has been a significant roadblock in developing effective treatment regimens, although existing therapeutics have yielded some degree of success. As a result, epigenetic signatures in EC have been the focus of biomarker research, offering the opportunity to serve not only in a diagnostic capacity, but also for the monitoring and prognostication of disease progression (progression markers), survival prognosis (prognostic markers), and likelihood of response to therapy (predictive markers). From scrutinizing methylomic changes in EC, several genes that present aberrant expression levels have been identified as preliminary inducers of carcinogenesis from BE to EAC, and can be seen before malignant histologic changes are observed [52]. Moreover, as the degree of DNA methylation becomes more prominent throughout the course of disease progression, clinicians are then able to more effectively implement an appropriate treatment plan that is specifically tailored to each patient.

While the primary focus of methylation markers in EC has been for surveillance purposes,

Table 1. Comparison of the commonly used screen methods for early esophageal carcinomas

METHOD	PROCEDURE	BENEFITS	DRAWBACKS	REF.
WHITE-LIGHT ENDOSCOPY	Endoscope with high-resolution imaging - may be done with endoluminal biopsy	Is sensitive enough to detect BE-LGD	Can't detect ESCC precursor lesions; expensive; invasive	[116]
LUGOL CHROMOENDOSCOPY	Application of colored dyes to esophagus for targeted biopsy	Inexpensive; high sensitivity	Inconsistent specificity for squamous dysplasia	[117]
TRANSNASAL ENDOSCOPY	Nasal esophageal intubation with optic scope	Economical; less invasive; no need for general anesthesia	Conscious sedation poses increased risk for complication	[118]
MICROENDOSCOPY	Confocal laser endomicroscopy of targeted biopsy tissue	Higher sensitivity and specificity, relative to conventional microscopy methods	Not economically feasible - can cost thousands of dollars	[119]
ENDOCYSTOCOPY	Ultra-magnification of stained, or in-vivo, epithelial tissue	High sensitivity/specificity	Not readily available; expensive; lack of standardized criteria for diagnosis leads to ambiguous results	[120]
CYTOSPONGE	Ingested sponge capsule is retrieved from patient after 5 minutes wherein the apparatus scrapes cells from the epithelium	Very high sensitivity/specificity; non-invasive; inexpensive; convenient; can collect 500,000 cells for epigenetic analysis therein increasing reproducibility and robustness	Still requires traditional endoscopic methods, following a positive result; sensitivity is still not 100%	[90]
ESOPHACAP	Ingested sponge is retrieved after several minutes whereupon removal, cells are extracted	Highest sensitivity/specificity; 1,000,000 cells are collected; inexpensive; convenient; non-invasive	Still requires traditional endoscopic methods, following a positive result	[91]

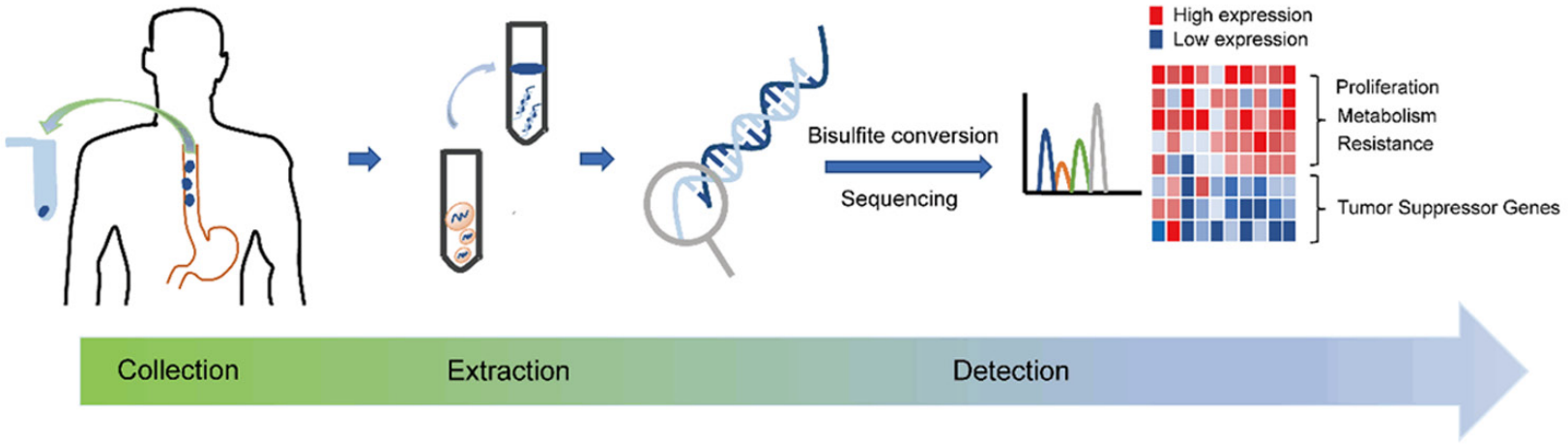

Figure 2. Methylation-based screening for esophageal carcinoma. Tissue biopsy can be obtained by endoscopic methods. After sample collection, it will be directly used for DNA extraction and bisulfite conversion, which could highlight the presence and location of DNA methylation. Several assays can be performed for methylation detection, such as sanger-sequencing or pyrosequencing.

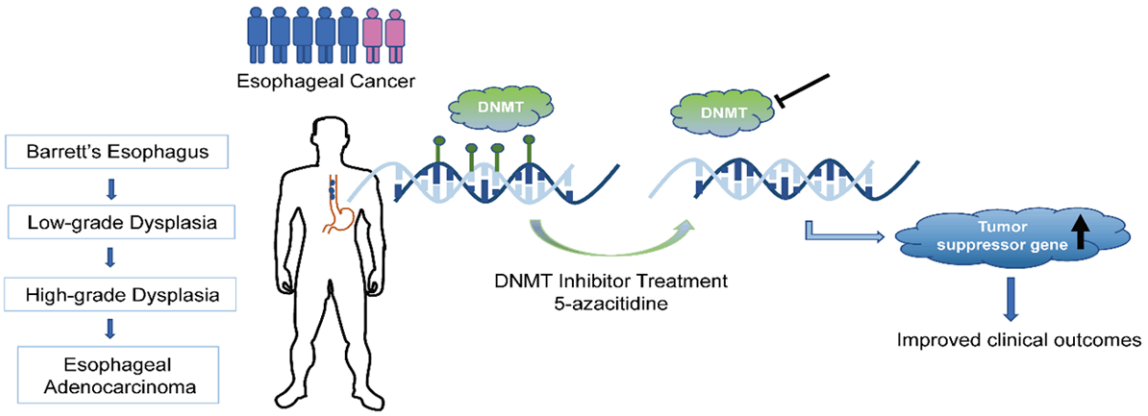

Figure 3. Application of DNMT inhibitor therapeutics in halting progression of EAC. During the progression of esophageal carcinoma, DNA methylation, catalyzed by DNMT, was modified on several genes, causing aberrant expression and activity. DNMT inhibitor treatment could reduce the degree/extent of DNA methylation and increase the expression of tumor suppressor genes, and thus improve clinical outcomes.

the stratification of EC's based upon distinct molecular signatures has shown promise in working towards delivering targeted therapeutics. Specifically, this classification of ECs into categorical subtypes has allowed clinicians to treat ESCC and EAC by addressing the root cause of abnormal gene activity [53]. For example, hypermethylated carcinomas appear to be sensitive to treatment with DNA methyltransferase and topoisomerase I inhibitors, while global hypomethylation may be sensitive to treatment with CDK2 inhibitors [54]. Although promising, further rigorous clinical validation is necessary to confirm the reliability of universal epigenetic alterations that are indicative of EC and its subsequent progression.

Ongoing methylation marker discovery and validation for esophageal carcinoma and precursor lesions

From data compiled by The Cancer Genome Atlas (TCGA) team, over 8,300 genes were found to be mutated across 165 cases of EAC [7]. Moreover, Bandla et al. reported the significance of mutational load in differentiating EAC from its precursor, BE [55]. Specifically, the buildup of epigenetic and genetic modifications in several genes - including *TP53*, *CDKN2A*, *CTNNB1*, and *APC* - that are commonly seen in EAC have also been reported to be present in BE, albeit less frequently [56-61]. The progressive accumulation of these mutations has therefore allowed for the stratification of disease into stages; this multistep progression starts with BE, then transitioning to low-grade dysplasia (LGD), subsequent high-grade dysplasia (HGD), and ultimately EAC [62, 63] (**Figure 3**). However, given the level of heterogeneity and complexity in EC, a meticulous validation process is essential for confirming the robustness of molecular signatures which are indicative of the presence of ECs.

In an effort to address this, Alvi et al. used an array-based approach and formed a four-marker panel (*PIGR*, *RIN2*, *GJA12*, and *SLC22A18*) that was able to distinguish between the presence of HGD/EAC and BE, and enhanced classification of disease-related risk, based upon the degree of methylation in this panel [64]. In a retrospective study, using a cohort consisting of 60 BE patients, 36 patients with dysplastic BE, and 90 with HGD/early EAC, this panel was validated by pyrosequencing and was shown to have a specificity of 97% and a sensitivity of 94%. In a broader approach, the Esophageal Cancer Clinical and Molecular Stratification (OCCAMS) consortium analyzed the results from whole-genome sequencing of 129 EAC cases and were able to categorize the mutational profiles into 3 distinct EAC groups based upon differing genomic mutations. These findings were substantiated in a subsequent study which analyzed the mutational profiles of another cohort, consisting of 87 patients [65]. This study served as the basis for Jammula et al.'s epigenetic analyses on over 300 BE and EAC cases; the results of which were consolidated with corresponding genomic and tran-

Table 2. Promising Epigenomic Biomarkers for EC

Marker	Function	Methylation Status	Malignancy	Reference
HER2 (ERBB2)	Cell differentiation; proliferation; suppresses apoptosis	Hypo	EAC	[110]
PD-L1	Immune checkpoint, signals for apoptosis	Hypo	ESCC	[111]
CDKN2A	Tumor suppressor gene	Hyper	EAC/ESCC	[73]
ABCD1	ABC transporter protein for fatty acids	Hypo	ESCC	[88]
SPIN3	Tumor suppressor gene	Hyper	ESCC	[88]
TP63	Antagonist of pro-apoptotic genes	Hypo	ESCC	[87]
GATA4/6	Transcription factor	Hypo	EAC	[87]
SOX2	Transcription factor; cell renewal	Hypo	ESCC	[87]
LINE-1	Transposable element	Hypo	ESCC	[99]
KRAS	Part of RAS/MAPK pathway; cell proliferation	Hypo	EAC/ESCC	[84]

scriptomic profiles to present a comprehensive analysis to confidently discern BE from EAC [54]. The data gathered allowed for the classification of EAC and BE tissues to be stratified into 4 subtypes; among the 4 subtypes, 3 involve aberrant methylation patterns that have been suggested to be clinically relevant for treatment regimen planning. Subtype 1 tissue displayed hypermethylation in the CpG islands of noncoding regions for genes necessary for DNA repair. Subtype 2 tissue likewise exhibited hypermethylation; however, also displayed hypomethylation in genes required for cell metabolism and ATP synthesis. The last subtype involving anomalous methylation signatures, subtype 4, presented extensive hypomethylation which resulted in significant structural variation.

Although having different etiologies, many of the aberrant methylation signatures found in EAC are also commonly seen in ESCC, further compounding the level of difficulty in biomarker validation. Moreover, ESCC presents the same multistep progression as is seen in EAC (non-dysplastic, LGD, HGD, carcinoma), wherein mutational load and accumulation of epigenetic alterations can assist with risk stratification for survivability and probability of carcinogenesis [66-71]. An example of this is the promoter hypermethylation of several tumor suppressor genes and DNA repair genes (*MGMT, p14, p16*) that have been found to be present in EAC as well as in dysplastic, ESCC precursor lesions [72-74]. Specifically, *CDKN2A* (official symbol for *p16*) hypermethylation is found to be present in up to 88% of ESCC tumors, and is indicative of an invasive phenotype [75-83]. Other well-known oncogenes such as *KRAS*, *IGF1R*, *CDK6*, and *EGFR* are also overexpressed in both EAC and ESCC, at similar frequencies. Additionally, the proximity in which these two malignancies can present, within the esophageal tract, may hinder the ability to accurately discern between histopathological differences.

To better discriminate between carcinomas in adjacent tissue, Pu et al. discovered and validated a 5-marker panel that showed significant CpG island hypermethylation in ESCC tissue, relative to EAC tissue. Three of these markers are in the promoter region of genes (*STK3*, *ZNF418*, and *ZNF542*) [84, 85]. Additionally, Agrawal et al. highlighted distinct spectra in C:G>T:A mutation profiles in EACs (46%) vs. ESCCs (35%), with A:T>C:G substitutions more commonly found in EAC as opposed to a higher incidence of C:G>G:C substitutions and indels found in ESCC [86]. A more recent molecular distinction was shown in the overexpression of several genes, namely *SOX2, CCND1,* and/or *TP63* are more frequently seen in ESCC, while *GATA6, GATA4,* and *ERBB2* are typically representative of EAC [87]. In a different study, Lu et al. analyzed RNA-seq and methylation profiles in ESCC and found five candidate genes (*ZNF608*, *SLC5A10*, *ZNF69*, *SPIN3*, and *ABCD1*) with aberrant methylomic signatures that may potentially serve as prognostic makers for ESCC (See **Table 2**) [88]. Furthermore, as more molecular markers for EC are being discovered and validated, aberrant DNA methylation is a promising method by which both EAC and ESCC can be not only accurately diagnosed, but further categorized based upon a host of factors affecting clinical outcomes.

Benefits and limitations of methylation markers in the detection and surveillance of EC

Although more progress still needs to be made, integrating methylation markers into routine EC screening has numerous potential benefits, including the potential for increased sensitivity and specificity over current methods [89]. Additionally, methylation marker panels that are currently being studied allow for the utilization of non-invasive methods of sample collection. In 2018, Chettouh et al. used a four-marker panel comprised of *TFPI2*, *TWIST1*, *ZNF569*, and *ZNF345* to screen for BE, and were able to use an inexpensive, non-endoscopic Cytosponge collection apparatus [90]. Moreover, Wang et al. used a non-invasive Esophacap to collect esophageal mucosal cells and were able to discern healthy controls from BE samples using a five-marker panel (AKAP12, NELL1, HPP1, p16, and TAC1) with a specificity of 92.8% and a sensitivity of 78.6% [91]. Given the potential of reduced costs, reduced need for invasive endoscopic procedures, and increased sensitivity and specificity, the implementation of robust methylation-marker panels for the detection and surveillance of ECs shows promise as an alternative to conventional methods.

On the other hand, in spite of the progress made in identifying and validating methylation markers in EC, current methodologies also have some limitations. Of note, there is interobserver variability in diagnosing BE-LGD and its subsequent progression to carcinoma, which makes it difficult to standardize the results of biomarker studies and to identify a potential universal methylation marker panel. To help address this issue, investigation of the epigenomic alterations in esophageal cells during BE and EC carcinogenesis has shown increasing promise as a method through which objective analysis can be conducted. In doing so, many have used in-vitro cell, and 3D, culture models and in vivo animal models; however, these studies have received criticism for large discrepancies between these models and human EC pathobiology [92-98]. In addition to interobserver variations among clinicians and scientists, the exceptional degree of heterogeneity seen in ECs further complicates the task of finding reliable indicators of malignancy, or precursor lesions. For example, hypomethylation of the repetitive long interspersed transposable element (LINE), LINE-1, has been proposed as a biomarker for ESCC that is strongly correlated with high risk for progression and poor prognosis. However, studies on LINE-1 in EC vary greatly in their analyses of the proportion of hypomethylation required (25-92% increase) to exercise deleterious affect [99-102]. Therefore, it is paramount that improvements in methylation biomarkers be made before being put into practice.

Current directions in biomarker-related treatments for EC

Although many therapeutic interventions for EC are either still in development or have yielded modest success, new insight into EC methylomes have provided potential therapeutic targets. Of growing interest, the employment of DNMT inhibitors has shown promise in combination therapies for the treatment of chemoresistant tumors. Specifically, the methylation-induced repression of specific genes, that would otherwise sensitize tumor cells to chemotherapy, nulls the cytotoxic effects of conventional therapies, thereby rendering these treatments ineffective. Additionally, as previously discussed, many tumor suppressor genes (TSGs) are hypermethylated during EC carcinogenesis. This epigenetic repression has consequently led to recent investigation into the application of DNMT inhibitors (**Figure 3**).

Currently, the most well-known DNMT inhibitor, albeit still not fully understood, is 5-azacitidine which is an FDA approved nucleoside analog that is capable of inducing hypomethylation and has been shown to improve EC patient survivability [103, 104]. In a cohort of 12 esophageal/gastric adenocarcinoma patients, Schneider et al. found that treatment with 5-azacitidine (V, 75 mg/m^2) for 3-5 days prior to chemotherapy resulted in reactivation of hypermethylated TSGs, which may result in hypomethylation-induced chemoresensitization, ultimately improving clinical outcomes of subsequent chemotherapy and resection of residual tumors [105]. In a similar study, Fu et al. found 5-azacitidine (V, 75 mg/m^2) for five days at least partially restored chemotherapeutic efficacy in chemoresistant ovarian cancers [106]. In addition to 5-azacitidine, the other FDA approved DNMT inhibitor is decitabine, the deoxy derivative of 5-azacitidine, which yields comparable results and works in

similar fashion: incorporating itself onto DNMT DNA strands during replication wherein it acts as a chain terminator to inhibit activity [107-109]. Although there are currently not many therapeutics that directly act on the epigenomic changes caused by EC carcinogenesis, advancements in EC biology and epigenomics have allowed clinicians to better formulate personalized treatment regimens using current therapeutics.

Aside from the direct therapeutic targeting of epigenetic writers, significant advancements in biomarker-based immunotherapy have been instrumental in directing future research efforts. In particular, excessive hypomethylation of well-known biomarkers, such as *PD-L1* and *HER2* have contributed to their overexpression within the tumor microenvironment, and thus, its immunosuppressive properties [110, 111]. Although further research needs to be done to improve efficacy, clinical trials on Trastuzumab, a humanized HER-2 monoclonal antibody, have improved progression-free survival in EAC patients, when combined with chemotherapy [112, 113]. Moreover, myriad studies and clinical trials on the efficacy of a PD-L1 blockade therapy, such as Nivolumab and Keytruda, have yielded objective response rates of up to 20%, adding as much as 6 months to overall survival in ESCC patients [114, 115]. It was also noted that overexpression of PD-L1 is correlated with microsatellite instability (MSI) and a DNA mismatch repair (MMR) deficiency. As a result, in conjunction with the PD-L1 and HER2 biomarkers, the National Comprehensive Cancer Network (NCCN) has also listed surveillance of MSI/MMR status to its pathologic biomarker testing guidelines. As previously mentioned, extensive DNA hypomethylation of centromeric and pericentromeric satellite sequences have significant deleterious effects on chromosomal stability and sequence integrity. Therefore, the combination of these suggested biomarkers, per NCCN guidelines, can serve in both diagnostic and therapeutic capacities, and are promising advancements in biomarker research for EC.

Conclusion

EC is one of the deadliest forms of cancer, with 5-year survival rate of less than 20%. This is largely due to asymptomatic patients who remain undiagnosed until late-stage carcinoma, when the survival rate is exceedingly low. This review addresses the significance of aberrant DNA methylation in ECs and the potential impact of advancing our understanding of methylation markers throughout the various stages of EC carcinogenesis, from non-dysplastic precursor lesions to invasive carcinoma. Moreover, studies over the past decade have revealed the applications of methylation markers in EC to be beneficial in diagnostic, prognostic, and therapeutic capacities. Of growing interest is the combination of distinct epigenomic markers to form “panels”, therein enhancing the accuracy of diagnostic and prognostic screening. Further, such panels have been helpful, thus far, in differentiating between different malignancies that would otherwise be difficult to discern due to proximity or histological presentation (See **Table 2**). Therefore, advancements in this field may not only improve overall survival, due to early detection, but may ultimately reveal novel therapeutic targets for improved treatment. However, the fact remains that despite improvements in methylome-based approaches for the detection, surveillance, and therapeutics for EC, challenges such as overcoming extensive heterogeneity and interobserver variability remain and underscore the importance of continuing to better understand DNA methylation in EC and to work towards improving this platform.

Disclosure of conflict of interest

None.

Address correspondence to: Zhongren Zhou, Department of Pathology & Laboratory Medicine, Rutgers-Robert Wood Johnson Medical School, Rutgers The State University of New Jersey, New Brunswick, NJ, United States. E-mail: zz442@rwjms.rutgers.edu; He Wang, Department of Pathology, Yale School of Medicine, Yale University, New Haven, Connecticut, United States. E-mail: he.wang@yale.edu

References

[1] Njei B, McCarty TR and Birk JW. Trends in esophageal cancer survival in United States adults from 1973 to 2009: a SEER database analysis. J Gastroenterol Hepatol 2016; 31: 1141-1146.

[2] Pohl H, Sirovich B and Welch HG. Esophageal adenocarcinoma incidence: are we reaching the peak? Cancer Epidemiol Biomarkers Prev 2010; 19: 1468-1470.

[3] Uhlenhopp DJ, Then EO, Sunkara T and Gaduputi V. Epidemiology of esophageal cancer: update in global trends, etiology and risk factors. Clin J Gastroenterol 2020; 13: 1010-1021.
[4] Arnold M, Colquhoun A, Cook MB, Ferlay J, Forman D and Soerjomataram I. Obesity and the incidence of upper gastrointestinal cancers: an ecological approach to examine differences across age and sex. Cancer Epidemiol Biomarkers Prev 2016; 25: 90-97.
[5] Zhang Y. Epidemiology of esophageal cancer. World J Gastroenterol 2013; 19: 5598-5606.
[6] Ilson DH and van Hillegersberg R. Management of patients with adenocarcinoma or squamous cancer of the esophagus. Gastroenterology 2018; 154: 437-451.
[7] Dulak AM, Stojanov P, Peng S, Lawrence MS, Fox C, Stewart C, Bandla S, Imamura Y, Schumacher SE, Shefler E, McKenna A, Carter SL, Cibulskis K, Sivachenko A, Saksena G, Voet D, Ramos AH, Auclair D, Thompson K, Sougnez C, Onofrio RC, Guiducci C, Beroukhim R, Zhou Z, Lin L, Lin J, Reddy R, Chang A, Landrenau R, Pennathur A, Ogino S, Luketich JD, Golub TR, Gabriel SB, Lander ES, Beer DG, Godfrey TE, Getz G and Bass AJ. Exome and whole-genome sequencing of esophageal adenocarcinoma identifies recurrent driver events and mutational complexity. Nat Genet 2013; 45: 478-486.
[8] Kaz AM, Grady WM, Stachler MD and Bass AJ. Genetic and epigenetic alterations in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterol Clin North Am 2015; 44: 473-489.
[9] Junker JP and van Oudenaarden A. Every cell is special: genome-wide studies add a new dimension to single-cell biology. Cell 2014; 157: 8-11.
[10] Li X, Francies HE, Secrier M, Perner J, Miremadi A, Galeano-Dalmau N, Barendt WJ, Letchford L, Leyden GM, Goffin EK, Barthorpe A, Lightfoot H, Chen E, Gilbert J, Noorani A, Devonshire G, Bower L, Grantham A, MacRae S, Grehan N, Wedge DC, Fitzgerald RC and Garnett MJ. Organoid cultures recapitulate esophageal adenocarcinoma heterogeneity providing a model for clonality studies and precision therapeutics. Nat Commun 2018; 9: 2983.
[11] Pectasides E, Stachler MD, Derks S, Liu Y, Maron S, Islam M, Alpert L, Kwak H, Kindler H, Polite B, Sharma MR, Allen K, O'Day E, Lomnicki S, Maranto M, Kanteti R, Fitzpatrick C, Weber C, Setia N, Xiao SY, Hart J, Nagy RJ, Kim KM, Choi MG, Min BH, Nason KS, O'Keefe L, Watanabe M, Baba H, Lanman R, Agoston AT, Oh DJ, Dunford A, Thorner AR, Ducar MD, Wollison BM, Coleman HA, Ji Y, Posner MC, Roggin K, Turaga K, Chang P, Hogarth K, Siddiqui U, Gelrud A, Ha G, Freeman SS, Rhoades J, Reed S, Gydush G, Rotem D, Davison J, Imamura Y, Adalsteinsson V, Lee J, Bass AJ and Catenacci DV. Genomic heterogeneity as a barrier to precision medicine in gastroesophageal adenocarcinoma. Cancer Discov 2018; 8: 37-48.
[12] Biswas S and Rao CM. Epigenetic tools (The Writers, The Readers and The Erasers) and their implications in cancer therapy. Eur J Pharmacol 2018; 837: 8-24.
[13] Moore LD, Le T and Fan G. DNA methylation and its basic function. Neuropsychopharmacology 2013; 38: 23-38.
[14] Suzuki MM and Bird A. DNA methylation landscapes: provocative insights from epigenomics. Nat Rev Genet 2008; 9: 465-476.
[15] Robertson KD. DNA methylation and human disease. Nat Rev Genet 2005; 6: 597-610.
[16] Ahrens TD, Werner M and Lassmann S. Epigenetics in esophageal cancers. Cell Tissue Res 2014; 356: 643-655.
[17] Baba Y, Watanabe M and Baba H. Review of the alterations in DNA methylation in esophageal squamous cell carcinoma. Surg Today 2013; 43: 1355-1364.
[18] Bedford MT and van Helden PD. Hypomethylation of DNA in pathological conditions of the human prostate. Cancer Res 1987; 47: 5274-5276.
[19] de Capoa A, Musolino A, Della Rosa S, Caiafa P, Mariani L, Del Nonno F, Vocaturo A, Donnorso RP, Niveleau A and Grappelli C. DNA demethylation is directly related to tumour progression: evidence in normal, pre-malignant and malignant cells from uterine cervix samples. Oncol Rep 2003; 10: 545-549.
[20] Brothman AR, Swanson G, Maxwell TM, Cui J, Murphy KJ, Herrick J, Speights VO, Isaac J and Rohr LR. Global hypomethylation is common in prostate cancer cells: a quantitative predictor for clinical outcome? Cancer Genet Cytogenet 2005; 156: 31-36.
[21] Cadieux B, Ching TT, VandenBerg SR and Costello JF. Genome-wide hypomethylation in human glioblastomas associated with specific copy number alteration, methylenetetrahydrofolate reductase allele status, and increased proliferation. Cancer Res 2006; 66: 8469-8476.
[22] Ehrlich M. DNA methylation in cancer: too much, but also too little. Oncogene 2002; 21: 5400-5413.
[23] Narayan A, Ji W, Zhang XY, Marrogi A, Graff JR, Baylin SB and Ehrlich M. Hypomethylation of pericentromeric DNA in breast adenocarcinomas. Int J Cancer 1998; 77: 833-838.
[24] Smith IM, Mydlarz WK, Mithani SK and Califano JA. DNA global hypomethylation in squamous cell head and neck cancer associated

with smoking, alcohol consumption and stage. Int J Cancer 2007; 121: 1724-1728.
[25] Baylin SB and Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet 2000; 16: 168-174.
[26] Belinsky SA, Nikula KJ, Palmisano WA, Michels R, Saccomanno G, Gabrielson E, Baylin SB and Herman JG. Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. Proc Natl Acad Sci U S A 1998; 95: 11891-11896.
[27] Wong DJ, Foster SA, Galloway DA and Reid BJ. Progressive region-specific de novo methylation of the p16 CpG island in primary human mammary epithelial cell strains during escape from M(0) growth arrest. Mol Cell Biol 1999; 19: 5642-5651.
[28] Nuovo GJ, Plaia TW, Belinsky SA, Baylin SB and Herman JG. In situ detection of the hypermethylation-induced inactivation of the p16 gene as an early event in oncogenesis. Proc Natl Acad Sci U S A 1999; 96: 12754-12759.
[29] Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA and Klingelhutz AJ. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. Nature 1998; 396: 84-88.
[30] Shen JC, Rideout WM 3rd and Jones PA. High frequency mutagenesis by a DNA methyltransferase. Cell 1992; 71: 1073-1080.
[31] Vinagre J, Almeida A, Populo H, Batista R, Lyra J, Pinto V, Coelho R, Celestino R, Prazeres H, Lima L, Melo M, da Rocha AG, Preto A, Castro P, Castro L, Pardal F, Lopes JM, Santos LL, Reis RM, Cameselle-Teijeiro J, Sobrinho-Simoes M, Lima J, Maximo V and Soares P. Frequency of TERT promoter mutations in human cancers. Nat Commun 2013; 4: 2185.
[32] Lee DD, Leao R, Komosa M, Gallo M, Zhang CH, Lipman T, Remke M, Heidari A, Nunes NM, Apolonio JD, Price AJ, De Mello RA, Dias JS, Huntsman D, Hermanns T, Wild PJ, Vanner R, Zadeh G, Karamchandani J, Das S, Taylor MD, Hawkins CE, Wasserman JD, Figueiredo A, Hamilton RJ, Minden MD, Wani K, Diplas B, Yan H, Aldape K, Akbari MR, Danesh A, Pugh TJ, Dirks PB, Castelo-Branco P and Tabori U. DNA hypermethylation within TERT promoter upregulates TERT expression in cancer. J Clin Invest 2019; 129: 223-229.
[33] Horn S, Figl A, Rachakonda PS, Fischer C, Sucker A, Gast A, Kadel S, Moll I, Nagore E, Hemminki K, Schadendorf D and Kumar R. TERT promoter mutations in familial and sporadic melanoma. Science 2013; 339: 959-961.
[34] Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L and Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science 2013; 339: 957-959.
[35] Iliopoulos D, Oikonomou P, Messinis I and Tsezou A. Correlation of promoter hypermethylation in hTERT, DAPK and MGMT genes with cervical oncogenesis progression. Oncol Rep 2009; 22: 199-204.
[36] de Wilde J, Kooter JM, Overmeer RM, Claassen-Kramer D, Meijer CJ, Snijders PJ and Steenbergen RD. hTERT promoter activity and CpG methylation in HPV-induced carcinogenesis. BMC Cancer 2010; 10: 271.
[37] Kumari A, Srinivasan R, Vasishta RK and Wig JD. Positive regulation of human telomerase reverse transcriptase gene expression and telomerase activity by DNA methylation in pancreatic cancer. Ann Surg Oncol 2009; 16: 1051-1059.
[38] Wang Z, Xu J, Geng X and Zhang W. Analysis of DNA methylation status of the promoter of human telomerase reverse transcriptase in gastric carcinogenesis. Arch Med Res 2010; 41: 1-6.
[39] Petit T, Georges C, Jung GM, Borel C, Bronner G, Flesch H, Massard G, Velten M, Haegele P and Schraub S. Systematic esophageal endoscopy screening in patients previously treated for head and neck squamous-cell carcinoma. Ann Oncol 2001; 12: 643-646.
[40] Roshandel G, Nourouzi A, Pourshams A, Semnani S, Merat S and Khoshnia M. Endoscopic screening for esophageal squamous cell carcinoma. Arch Iran Med 2013; 16: 351-357.
[41] Carvalho R, Areia M, Brito D, Saraiva S, Alves S and Cadime AT. Diagnostic accuracy of lugol chromoendoscopy in the oesophagus in patients with head and neck cancer. Rev Esp Enferm Dig 2013; 105: 79-83.
[42] Dubuc J, Legoux J, Winnock M, Seyrig J, Barbier J, Barrioz T, Laugier R, Boulay G, Grasset D, Sautereau D, Grigoresco D, Butel J, Scoazec J and Ponchon T; Société Française d'Endoscopie Digestive. Endoscopic screening for esophageal squamous-cell carcinoma in high-risk patients: a prospective study conducted in 62 French endoscopy centers. Endoscopy 2006; 38: 690-695.
[43] Nagami Y, Tominaga K, Machida H, Nakatani M, Kameda N, Sugimori S, Okazaki H, Tanigawa T, Yamagami H, Kubo N, Shiba M, Watanabe K, Watanabe T, Iguchi H, Fujiwara Y, Ohira M, Hirakawa K and Arakawa T. Usefulness of non-magnifying narrow-band imaging in screening of early esophageal squamous cell carcinoma: a prospective comparative study using propensity score matching. Am J Gastroenterol 2014; 109: 845-854.
[44] Morita FH, Bernardo WM, Ide E, Rocha RS, Aquino JC, Minata MK, Yamazaki K, Marques

SB, Sakai P and de Moura EG. Narrow band imaging versus lugol chromoendoscopy to diagnose squamous cell carcinoma of the esophagus: a systematic review and meta-analysis. BMC Cancer 2017; 17: 54.

[45] Shames DS, Minna JD and Gazdar AF. Methods for detecting DNA methylation in tumors: from bench to bedside. Cancer Lett 2007; 251: 187-198.

[46] Frommer M, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, Molloy PL and Paul CL. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. Proc Natl Acad Sci U S A 1992; 89: 1827-1831.

[47] Gouil Q and Keniry A. Latest techniques to study DNA methylation. Essays Biochem 2019; 63: 639-648.

[48] Colella S, Shen L, Baggerly KA, Issa JP and Krahe R. Sensitive and quantitative universal pyrosequencing methylation analysis of CpG sites. Biotechniques 2003; 35: 146-150.

[49] Suzuki H, Gabrielson E, Chen W, Anbazhagan R, van Engeland M, Weijenberg MP, Herman JG and Baylin SB. A genomic screen for genes upregulated by demethylation and histone deacetylase inhibition in human colorectal cancer. Nat Genet 2002; 31: 141-149.

[50] Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL and Schubeler D. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nat Genet 2005; 37: 853-862.

[51] Feinberg AP and Tycko B. The history of cancer epigenetics. Nat Rev Cancer 2004; 4: 143-153.

[52] Kalatskaya I. Overview of major molecular alterations during progression from Barrett's esophagus to esophageal adenocarcinoma. Ann N Y Acad Sci 2016; 1381: 74-91.

[53] Gao YB, Chen ZL, Li JG, Hu XD, Shi XJ, Sun ZM, Zhang F, Zhao ZR, Li ZT, Liu ZY, Zhao YD, Sun J, Zhou CC, Yao R, Wang SY, Wang P, Sun N, Zhang BH, Dong JS, Yu Y, Luo M, Feng XL, Shi SS, Zhou F, Tan FW, Qiu B, Li N, Shao K, Zhang LJ, Zhang LJ, Xue Q, Gao SG and He J. Genetic landscape of esophageal squamous cell carcinoma. Nat Genet 2014; 46: 1097-1102.

[54] Jammula S, Katz-Summercorn AC, Li X, Linossi C, Smyth E, Killcoyne S, Biasci D, Subash VV, Abbas S, Blasko A, Devonshire G, Grantham A, Wronowski F, O'Donovan M, Grehan N, Eldridge MD and Tavaré S; Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) consortium, Fitzgerald RC. Identification of subtypes of Barrett's esophagus and esophageal adenocarcinoma based on DNA methylation profiles and integration of transcriptome and genome data. Gastroenterology 2020; 158: 1682-1697, e1681.

[55] Bandla S, Peters JH, Ruff D, Chen SM, Li CY, Song K, Thoms K, Litle VR, Watson T, Chapurin N, Lada M, Pennathur A, Luketich JD, Peterson D, Dulak A, Lin L, Bass A, Beer DG, Godfrey TE and Zhou Z. Comparison of cancer-associated genetic abnormalities in columnar-lined esophagus tissues with and without goblet cells. Ann Surg 2014; 260: 72-80.

[56] Galipeau PC, Prevo LJ, Sanchez CA, Longton GM and Reid BJ. Clonal expansion and loss of heterozygosity at chromosomes 9p and 17p in premalignant esophageal (Barrett's) tissue. J Natl Cancer Inst 1999; 91: 2087-2095.

[57] Maley CC, Galipeau PC, Li X, Sanchez CA, Paulson TG, Blount PL and Reid BJ. The combination of genetic instability and clonal expansion predicts progression to esophageal adenocarcinoma. Cancer Res 2004; 64: 7629-7633.

[58] Maley CC, Galipeau PC, Finley JC, Wongsurawat VJ, Li X, Sanchez CA, Paulson TG, Blount PL, Risques RA, Rabinovitch PS and Reid BJ. Genetic clonal diversity predicts progression to esophageal adenocarcinoma. Nat Genet 2006; 38: 468-473.

[59] Reid BJ, Li X, Galipeau PC and Vaughan TL. Barrett's oesophagus and oesophageal adenocarcinoma: time for a new synthesis. Nat Rev Cancer 2010; 10: 87-101.

[60] Li X, Galipeau PC, Sanchez CA, Blount PL, Maley CC, Arnaudo J, Peiffer DA, Pokholok D, Gunderson KL and Reid BJ. Single nucleotide polymorphism-based genome-wide chromosome copy change, loss of heterozygosity, and aneuploidy in Barrett's esophagus neoplastic progression. Cancer Prev Res (Phila) 2008; 1: 413-423.

[61] Kaz AM, Wong CJ, Luo Y, Virgin JB, Washington MK, Willis JE, Leidner RS, Chak A and Grady WM. DNA methylation profiling in Barrett's esophagus and esophageal adenocarcinoma reveals unique methylation signatures and molecular subclasses. Epigenetics 2011; 6: 1403-1412.

[62] Paulson TG and Reid BJ. Focus on Barrett's esophagus and esophageal adenocarcinoma. Cancer Cell 2004; 6: 11-16.

[63] Yu M, Maden SK, Stachler M, Kaz AM, Ayers J, Guo Y, Carter KT, Willbanks A, Heinzerling TJ, O'Leary RM, Xu X, Bass A, Chandar AK, Chak A, Elliott R, Willis JE, Markowitz SD and Grady WM. Subtypes of Barrett's oesophagus and oesophageal adenocarcinoma based on genome-wide methylation analysis. Gut 2019; 68: 389-399.

[64] Alvi MA, Liu X, O'Donovan M, Newton R, Wernisch L, Shannon NB, Shariff K, di Pietro M, Bergman JJ, Ragunath K and Fitzgerald RC.

DNA methylation as an adjunct to histopathology to detect prevalent, inconspicuous dysplasia and early-stage neoplasia in Barrett's esophagus. Clin Cancer Res 2013; 19: 878-888.

[65] Secrier M, Li X, de Silva N, Eldridge MD, Contino G, Bornschein J, MacRae S, Grehan N, O'Donovan M, Miremadi A, Yang TP, Bower L, Chettouh H, Crawte J, Galeano-Dalmau N, Grabowska A, Saunders J, Underwood T, Waddell N, Barbour AP, Nutzinger B, Achilleos A, Edwards PA, Lynch AG, Tavaré S and Fitzgerald RC; Oesophageal Cancer Clinical and Molecular Stratification (OCCAMS) Consortium. Mutational signatures in esophageal adenocarcinoma define etiologically distinct subgroups with therapeutic relevance. Nat Genet 2016; 48: 1131-1141.

[66] Lin L and Lin DC. Biological significance of tumor heterogeneity in esophageal squamous cell carcinoma. Cancers (Basel) 2019; 11: 1156.

[67] Lin DC, Wang MR and Koeffler HP. Genomic and epigenomic aberrations in esophageal squamous cell carcinoma and implications for patients. Gastroenterology 2018; 154: 374-389.

[68] Toh Y, Egashira A and Yamamoto M. Epigenetic alterations and their clinical implications in esophageal squamous cell carcinoma. Gen Thorac Cardiovasc Surg 2013; 61: 262-269.

[69] You JS and Jones PA. Cancer genetics and epigenetics: two sides of the same coin? Cancer Cell 2012; 22: 9-20.

[70] Sawan C, Vaissiere T, Murr R and Herceg Z. Epigenetic drivers and genetic passengers on the road to cancer. Mutat Res 2008; 642: 1-13.

[71] Teng H, Xue M, Liang J, Wang X, Wang L, Wei W, Li C, Zhang Z, Li Q, Ran X, Shi X, Cai W, Wang W, Gao H and Sun Z. Inter- and intratumor DNA methylation heterogeneity associated with lymph node metastasis and prognosis of esophageal squamous cell carcinoma. Theranostics 2020; 10: 3035-3048.

[72] Lin L, Cheng X and Yin D. Aberrant DNA methylation in esophageal squamous cell carcinoma: biological and clinical implications. Front Oncol 2020; 10: 549850.

[73] Li JS, Ying JM, Wang XW, Wang ZH, Tao Q and Li LL. Promoter methylation of tumor suppressor genes in esophageal squamous cell carcinoma. Chin J Cancer 2013; 32: 3-11.

[74] Lima SC, Hernandez-Vargas H, Simao T, Durand G, Kruel CD, Le Calvez-Kelm F, Ribeiro Pinto LF and Herceg Z. Identification of a DNA methylome signature of esophageal squamous cell carcinoma and potential epigenetic biomarkers. Epigenetics 2011; 6: 1217-1227.

[75] Guo M, Ren J, House MG, Qi Y, Brock MV and Herman JG. Accumulation of promoter methylation suggests epigenetic progression in squamous cell carcinoma of the esophagus. Clin Cancer Res 2006; 12: 4515-4522.

[76] Lee EJ, Lee BB, Han J, Cho EY, Shim YM, Park J and Kim DH. CpG island hypermethylation of E-cadherin (CDH1) and integrin alpha4 is associated with recurrence of early stage esophageal squamous cell carcinoma. Int J Cancer 2008; 123: 2073-2079.

[77] Fukuoka T, Hibi K and Nakao A. Aberrant methylation is frequently observed in advanced esophageal squamous cell carcinoma. Anticancer Res 2006; 26: 3333-3335.

[78] Wang J, Sasco AJ, Fu C, Xue H, Guo G, Hua Z, Zhou Q, Jiang Q and Xu B. Aberrant DNA methylation of P16, MGMT, and hMLH1 genes in combination with MTHFR C677T genetic polymorphism in esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev 2008; 17: 118-125.

[79] Ling Y, Huang G, Fan L, Wei L, Zhu J, Liu Y, Zhu C and Zhang C. CpG island methylator phenotype of cell-cycle regulators associated with TNM stage and poor prognosis in patients with oesophageal squamous cell carcinoma. J Clin Pathol 2011; 64: 246-251.

[80] Li B, Wang B, Niu LJ, Jiang L and Qiu CC. Hypermethylation of multiple tumor-related genes associated with DNMT3b up-regulation served as a biomarker for early diagnosis of esophageal squamous cell carcinoma. Epigenetics 2011; 6: 307-316.

[81] Taghavi N, Biramijamal F, Sotoudeh M, Khademi H, Malekzadeh R, Moaven O, Memar B, A'Rabi A and Abbaszadegan MR. p16INK4a hypermethylation and p53, p16 and MDM2 protein expression in esophageal squamous cell carcinoma. BMC Cancer 2010; 10: 138.

[82] Salam I, Hussain S, Mir MM, Dar NA, Abdullah S, Siddiqi MA, Lone RA, Zargar SA, Sharma S, Hedau S, Basir SF, Bharti AC and Das BC. Aberrant promoter methylation and reduced expression of p16 gene in esophageal squamous cell carcinoma from Kashmir valley: a high-risk area. Mol Cell Biochem 2009; 332: 51-58.

[83] Maesawa C, Tamura G, Nishizuka S, Ogasawara S, Ishida K, Terashima M, Sakata K, Sato N, Saito K and Satodate R. Inactivation of the CDKN2 gene by homozygous deletion and de novo methylation is associated with advanced stage esophageal squamous cell carcinoma. Cancer Res 1996; 56: 3875-3878.

[84] Pu W, Wang C, Chen S, Zhao D, Zhou Y, Ma Y, Wang Y, Li C, Huang Z, Jin L, Guo S, Wang J and Wang M. Targeted bisulfite sequencing identified a panel of DNA methylation-based biomarkers for esophageal squamous cell carcinoma (ESCC). Clin Epigenetics 2017; 9: 129.

[85] Li X, Zhou F, Jiang C, Wang Y, Lu Y, Yang F, Wang N, Yang H, Zheng Y and Zhang J. Identifi-

cation of a DNA methylome profile of esophageal squamous cell carcinoma and potential plasma epigenetic biomarkers for early diagnosis. PLoS One 2014; 9: e103162.

[86] Agrawal N, Jiao Y, Bettegowda C, Hutfless SM, Wang Y, David S, Cheng Y, Twaddell WS, Latt NL, Shin EJ, Wang LD, Wang L, Yang W, Velculescu VE, Vogelstein B, Papadopoulos N, Kinzler KW and Meltzer SJ. Comparative genomic analysis of esophageal adenocarcinoma and squamous cell carcinoma. Cancer Discov 2012; 2: 899-905.

[87] Cancer Genome Atlas Research Network; Analysis Working Group: Asan University; BC Cancer Agency; Brigham and Women's Hospital; Broad Institute; Brown University; Case Western Reserve University; Dana-Farber Cancer Institute; Duke University; Greater Poland Cancer Centre; Harvard Medical School; Institute for Systems Biology; KU Leuven; Mayo Clinic; Memorial Sloan Kettering Cancer Center; National Cancer Institute; Nationwide Children's Hospital; Stanford University; University of Alabama; University of Michigan; University of North Carolina; University of Pittsburgh; University of Rochester; University of Southern California; University of Texas MD Anderson Cancer Center; University of Washington; Van Andel Research Institute; Vanderbilt University; Washington University; Genome Sequencing Center: Broad Institute; Washington University in St. Louis; Genome Characterization Centers: BC Cancer Agency; Broad Institute; Harvard Medical School; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University; University of North Carolina; University of Southern California Epigenome Center; University of Texas MD Anderson Cancer Center; Van Andel Research Institute; Genome Data Analysis Centers: Broad Institute; Brown University:; Harvard Medical School; Institute for Systems Biology; Memorial Sloan Kettering Cancer Center; University of California Santa Cruz; University of Texas MD Anderson Cancer Center; Biospecimen Core Resource: International Genomics Consortium; Research Institute at Nationwide Children's Hospital; Tissue Source Sites: Analytic Biologic Services; Asan Medical Center; Asterand Bioscience; Barretos Cancer Hospital; BioreclamationIVT; Botkin Municipal Clinic; Chonnam National University Medical School; Christiana Care Health System; Cureline; Duke University; Emory University; Erasmus University; Indiana University School of Medicine; Institute of Oncology of Moldova; International Genomics Consortium; Invidumed; Israelitisches Krankenhaus Hamburg; Keimyung University School of Medicine; Memorial Sloan Kettering Cancer Center; National Cancer Center Goyang; Ontario Tumour Bank; Peter MacCallum Cancer Centre; Pusan National University Medical School; Ribeirão Preto Medical School; St. Joseph's Hospital &Medical Center; St. Petersburg Academic University; Tayside Tissue Bank; University of Dundee; University of Kansas Medical Center; University of Michigan; University of North Carolina at Chapel Hill; University of Pittsburgh School of Medicine; University of Texas MD Anderson Cancer Center; Disease Working Group: Duke University; Memorial Sloan Kettering Cancer Center; National Cancer Institute; University of Texas MD Anderson Cancer Center; Yonsei University College of Medicine; Data Coordination Center: CSRA Inc.; Project Team: National Institutes of Health. Integrated genomic characterization of oesophageal carcinoma. Nature 2017; 541: 169-175.

[88] Lu T, Chen D, Wang Y, Sun X, Li S, Miao S, Wo Y, Dong Y, Leng X, Du W and Jiao W. Identification of DNA methylation-driven genes in esophageal squamous cell carcinoma: a study based on The Cancer Genome Atlas. Cancer Cell Int 2019; 19: 52.

[89] Codipilly DC, Qin Y, Dawsey SM, Kisiel J, Topazian M, Ahlquist D and Iyer PG. Screening for esophageal squamous cell carcinoma: recent advances. Gastrointest Endosc 2018; 88: 413-426.

[90] Chettouh H, Mowforth O, Galeano-Dalmau N, Bezawada N, Ross-Innes C, MacRae S, Debiram-Beecham I, O'Donovan M and Fitzgerald RC. Methylation panel is a diagnostic biomarker for Barrett's oesophagus in endoscopic biopsies and non-endoscopic cytology specimens. Gut 2018; 67: 1942-1949.

[91] Wang Z, Kambhampati S, Cheng Y, Ma K, Simsek C, Tieu AH, Abraham JM, Liu X, Prasath V, Duncan M, Stark A, Trick A, Tsai HL, Wang H, He Y, Khashab MA, Ngamruengphong S, Shin EJ, Wang TH and Meltzer SJ. Methylation biomarker panel performance in esophacap cytology samples for diagnosing Barrett's esophagus: a prospective validation study. Clin Cancer Res 2019; 25: 2127-2135.

[92] Bus P, Siersema PD and van Baal JW. Cell culture models for studying the development of Barrett's esophagus: a systematic review. Cell Oncol (Dordr) 2012; 35: 149-161.

[93] Das KM, Kong Y, Bajpai M, Kulkarni D, Geng X, Mishra P, Banerjee D and Hirshfield K. Transformation of benign Barrett's epithelium by repeated acid and bile exposure over 65 weeks: a novel in vitro model. Int J Cancer 2011; 128: 274-282.

[94] Minacapelli CD, Bajpai M, Geng X, Cheng CL, Chouthai AA, Souza R, Spechler SJ and Das KM. Barrett's metaplasia develops from cellu-

lar reprograming of esophageal squamous epithelium due to gastroesophageal reflux. Am J Physiol Gastrointest Liver Physiol 2017; 312: G615-G622.
[95] Whelan KA, Muir AB and Nakagawa H. Esophageal 3D culture systems as modeling tools in esophageal epithelial pathobiology and personalized medicine. Cell Mol Gastroenterol Hepatol 2018; 5: 461-478.
[96] Kapoor H, Lohani KR, Lee TH, Agrawal DK and Mittal SK. Animal models of Barrett's esophagus and esophageal adenocarcinoma-past, present, and future. Clin Transl Sci 2015; 8: 841-847.
[97] Nair DV and Reddy AG. Laboratory animal models for esophageal cancer. Vet World 2016; 9: 1229-1232.
[98] Jiang M, Li H, Zhang Y, Yang Y, Lu R, Liu K, Lin S, Lan X, Wang H, Wu H, Zhu J, Zhou Z, Xu J, Lee DK, Zhang L, Lee YC, Yuan J, Abrams JA, Wang TC, Sepulveda AR, Wu Q, Chen H, Sun X, She J, Chen X and Que J. Transitional basal cells at the squamous-columnar junction generate Barrett's oesophagus. Nature 2017; 550: 529-533.
[99] Iwagami S, Baba Y, Watanabe M, Shigaki H, Miyake K, Ishimoto T, Iwatsuki M, Sakamaki K, Ohashi Y and Baba H. LINE-1 hypomethylation is associated with a poor prognosis among patients with curatively resected esophageal squamous cell carcinoma. Ann Surg 2013; 257: 449-455.
[100] Kawano H, Saeki H, Kitao H, Tsuda Y, Otsu H, Ando K, Ito S, Egashira A, Oki E, Morita M, Oda Y and Maehara Y. Chromosomal instability associated with global DNA hypomethylation is associated with the initiation and progression of esophageal squamous cell carcinoma. Ann Surg Oncol 2014; 21 Suppl 4: S696-702.
[101] Hoshimoto S, Takeuchi H, Ono S, Sim MS, Huynh JL, Huang SK, Marzese DM, Kitagawa Y and Hoon DS. Genome-wide hypomethylation and specific tumor-related gene hypermethylation are associated with esophageal squamous cell carcinoma outcome. J Thorac Oncol 2015; 10: 509-517.
[102] Baba Y, Watanabe M, Murata A, Shigaki H, Miyake K, Ishimoto T, Iwatsuki M, Iwagami S, Yoshida N, Oki E, Sakamaki K, Nakao M and Baba H. LINE-1 hypomethylation, DNA copy number alterations, and CDK6 amplification in esophageal squamous cell carcinoma. Clin Cancer Res 2014; 20: 1114-1124.
[103] Jones PA and Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. Cell 1980; 20: 85-93.
[104] Issa JP, Kantarjian HM and Kirkpatrick P. Azacitidine. Nat Rev Drug Discov 2005; 4: 275-276.
[105] Schneider BJ, Shah MA, Klute K, Ocean A, Popa E, Altorki N, Lieberman M, Schreiner A, Yantiss R, Christos PJ, Palmer R, You D, Viale A, Kermani P and Scandura JM. Phase I study of epigenetic priming with azacitidine prior to standard neoadjuvant chemotherapy for patients with resectable gastric and esophageal adenocarcinoma: evidence of tumor hypomethylation as an indicator of major histopathologic response. Clin Cancer Res 2017; 23: 2673-2680.
[106] Fu S, Hu W, Iyer R, Kavanagh JJ, Coleman RL, Levenback CF, Sood AK, Wolf JK, Gershenson DM, Markman M, Hennessy BT, Kurzrock R and Bast RC Jr. Phase 1b-2a study to reverse platinum resistance through use of a hypomethylating agent, azacitidine, in patients with platinum-resistant or platinum-refractory epithelial ovarian cancer. Cancer 2011; 117: 1661-1669.
[107] Yang YM, Hong P, Xu WW, He QY and Li B. Advances in targeted therapy for esophageal cancer. Signal Transduct Target Ther 2020; 5: 229.
[108] Liao L, Yao ZT, Fang WK, He QY, Xu WW and Li B. Epigenetics in esophageal cancer: from mechanisms to therapeutics. Small Methods 2020; 4: 2000391.
[109] Chen M, Nie J, Liu Y, Li X, Zhang Y, Brock MV, Feng K, Wu Z, Li X, Shi L, Li S, Guo M, Mei Q and Han W. Phase Ib/II study of safety and efficacy of low-dose decitabine-primed chemoimmunotherapy in patients with drug-resistant relapsed/refractory alimentary tract cancer. Int J Cancer 2018; 143: 1530-1540.
[110] Battaglin F, Naseem M, Puccini A and Lenz HJ. Molecular biomarkers in gastro-esophageal cancer: recent developments, current trends and future directions. Cancer Cell Int 2018; 18: 99.
[111] Dong P, Xiong Y, Yue J, Hanley SJB and Watari H. Tumor-intrinsic PD-L1 signaling in cancer initiation, development and treatment: beyond immune evasion. Front Oncol 2018; 8: 386.
[112] Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J and Kang YK; ToGA Trial Investigators. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010; 376: 687-697.
[113] Press MF, Ellis CE, Gagnon RC, Grob TJ, Buyse M, Villalobos I, Liang Z, Wu S, Bang YJ, Qin SK, Chung HC, Xu J, Park JO, Jeziorski K, Afenjar K, Ma Y, Estrada MC, Robinson DM, Scherer SJ, Sauter G, Hecht JR and Slamon DJ. HER2 sta-

tus in advanced or metastatic gastric, esophageal, or gastroesophageal adenocarcinoma for entry to the TRIO-013/LOGiC trial of lapatinib. Mol Cancer Ther 2017; 16: 228-238.
[114] Kang YK, Boku N, Satoh T, Ryu MH, Chao Y, Kato K, Chung HC, Chen JS, Muro K, Kang WK, Yeh KH, Yoshikawa T, Oh SC, Bai LY, Tamura T, Lee KW, Hamamoto Y, Kim JG, Chin K, Oh DY, Minashi K, Cho JY, Tsuda M and Chen LT. Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens (ONO-4538-12, ATTRACTION-2): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2017; 390: 2461-2471.
[115] Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, Sun W, Jalal SI, Shah MA, Metges JP, Garrido M, Golan T, Mandala M, Wainberg ZA, Catenacci DV, Ohtsu A, Shitara K, Geva R, Bleeker J, Ko AH, Ku G, Philip P, Enzinger PC, Bang YJ, Levitan D, Wang J, Rosales M, Dalal RP and Yoon HH. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer: phase 2 clinical KEYNOTE-059 trial. JAMA Oncol 2018; 4: e180013.
[116] Yip HC and Chiu PW. Endoscopic diagnosis and management of early squamous cell carcinoma of esophagus. J Thorac Dis 2017; 9 Suppl 8: S689-S696.
[117] Li J, Xu R, Liu M, Cai H, Cao C, Liu F, Li F, Guo C, Pan Y, He Z and Ke Y. Lugol chromoendoscopy detects esophageal dysplasia with low levels of sensitivity in a high-risk region of China. Clin Gastroenterol Hepatol 2018; 16: 1585-1592.
[118] Chung EJ, Rho YS, Jung KY, Kim JW and Lee SW. The role of transnasal esophagoscopy in ent office: a prospective, multicenter study in Korea. Clin Exp Otorhinolaryngol 2014; 7: 123-125.
[119] Hur C, Choi SE, Kong CY, Wang GQ, Xu H, Polydorides AD, Xue LY, Perzan KE, Tramontano AC, Richards-Kortum RR and Anandasabapathy S. High-resolution microendoscopy for esophageal cancer screening in China: a cost-effectiveness analysis. World J Gastroenterol 2015; 21: 5513-5523.
[120] Abad MRA, Shimamura Y, Fujiyoshi Y, Seewald S and Inoue H. Endocytoscopy: technology and clinical application in upper gastrointestinal tract. Transl Gastroenterol Hepatol 2020; 5: 28.