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Mini-review

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.

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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 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 throughlow-grade

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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.

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 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.