

Epigenetics in the Pathogenesis of Esophageal Adenocarcinoma

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Abstract

Epigenetic influences, such as DNA methylation, histone acetylation, and up-regulation/down-regulation of genes by microRNAs, change the genetic makeup of an individual without affecting DNA base-pair sequences. Indeed, epigenetic changes play an integral role in the progression from normal esophageal mucosa to Barrett's esophagus to esophageal adenocarcinoma via dysplasia–metaplasia–neoplasia sequence. Many genes involved in esophageal adenocarcinoma display hypermethylation, leading to their down-regulation. The classes of these genes include cell cycle control, DNA and growth factor repair, tumor suppressors, antimetastasis, Wnt-related genes, and proapoptotic genes. Histone acetylation in the pathophysiology of esophageal diseases has not been thoroughly investigated, and its critical role in the development of esophageal adenocarcinoma is less defined. Many microRNAs have been associated with the development of Barrett's esophagus and esophageal adenocarcinoma. Here, we critically addressed the specific steps most closely influenced by microRNAs in the progression from Barrett's esophagus to esophageal adenocarcinoma. However, microRNAs can target up to hundreds of genes, making it difficult to correlate directly with a given phenotype of the disease. Esophageal adenocarcinoma progressing from premalignant condition of Barrett's esophagus carries an extremely poor prognosis. Risk stratification for patients based on their epigenetic profiles may be useful in providing more targeted and directed treatment to patients. *Clin Trans Sci* 2015; Volume 8: 394–402

Keywords: DNA methylation, epigenetics, Barrett's esophagus, esophageal adenocarcinoma, esophageal squamous cell carcinoma, histone acetylation, miRNA

Introduction

Esophageal cancer is the 8th most common cancer worldwide, and the fifth leading cause of cancer-related death in men.^{1,2} There are two main types of cancer that occur in the esophagus: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCC typically affects the upper 2/3rd of the esophagus, while EAC typically affects the lower third of the esophagus.³ The focus of this review article is esophageal adenocarcinoma. Rates of esophageal adenocarcinoma have been rising over the past four decades particularly in developed countries, and EAC carries a poor prognosis with roughly 25% of patients presenting with metastatic disease.⁴

Known risk factors for EAC include gastroesophageal reflux disease (GERD), Barrett's esophagus (BE), obesity, Caucasian race, increasing age, and smoking.^{5,6} It is believed that EAC develops through a metaplasia–dysplasia–neoplasia sequence as a result of chronic GERD.⁶ Long-standing GERD leads to Barrett's esophagus (*Figure 1*), a condition in which the normal squamous epithelium lining the esophagus is replaced by metaplastic columnar epithelium with intestinalization.⁷ Barrett's esophagus is a precancerous condition and is the only known precursor of esophageal adenocarcinoma.⁸ While the molecular mechanism of the transition into adenocarcinoma is not clear, it has been speculated that GERD may cause genetic and epigenetic changes in the epithelium leading to the characteristics seen in Barrett's esophagus and EAC.⁶

There is a lacunae in our understanding of the mechanisms underlying the progression of BE into EAC as only about 5% of patients with BE develop EAC. One study found that age might play a role in the development of familial versus nonfamilial Barrett's esophagus.⁹ Multiplex familial Barrett's esophagus was defined as having at least two family members with Barrett's esophagus.⁹ This familial aggregation could be caused by either common environmental exposures in family members or a genetic predisposition to the disease or both.⁹ However, the interpretation of these results is problematic because GERD is symptomatic and associated with the development of Barrett's esophagus,

while patients with BE may not be symptomatic.⁹ Therefore, it is difficult to establish the age of incidence.⁹ Endoscopic surveillance of Barrett's esophagus has not proven to be a very strong preventative measure of Barrett's esophagus, as only a small percentage (5%) of patients with EAC have a preexisting diagnosis of BE. Additionally, the rate of progression from BE to EAC is only 0.5% per year with a lifetime risk of 5%.⁹ Therefore, there is a great need for markers to predict which patients with GERD are at risk for developing BE and which patients with BE are at risk of developing EAC. This would allow for the prudent use of resources for screening and surveillance endoscopy, and it would potentially indicate the need for more aggressive ablative treatment of BE. The role of several biomarkers, including DNA methylation in Barrett's esophagus, has been explored. In this article, we critically reviewed the current status of biomarkers in the GERD → BE → EAC sequence and discussed a potential role of epigenetics in the pathogenesis and progression of the disease process. We also discussed the significance of this knowledge in developing biomarkers that correlate more closely with disease progression.

Genomic instability could be a critical factor to initiate the metaplasia–dysplasia–neoplasia sequence. Several studies have explored the role of *p53*, *APC*, *CDKN2A*, *cyclin D1*, and *Rb* genes. Mutations in these genes have been implicated in the progression of many cancers including EAC.¹⁰ While some genetic linkages have been found, epigenetics may be more helpful in predicting the progression of esophageal adenocarcinoma in patients with Barrett's esophagus. Epigenetics is defined as the “study of changes in gene function that are mitotically and/or meiotically heritable that do not entail a change in DNA sequence.”⁶ The three major processes in epigenetics are DNA methylation, histone acetylation/deacetylation, and miRNA.¹¹ DNA methylation involves modification of the DNA itself, whereas histone acetylation/deacetylation involves modification in the packaging of DNA, and miRNAs are short noncoding molecules that can alter gene expression.⁶ DNA methylation is

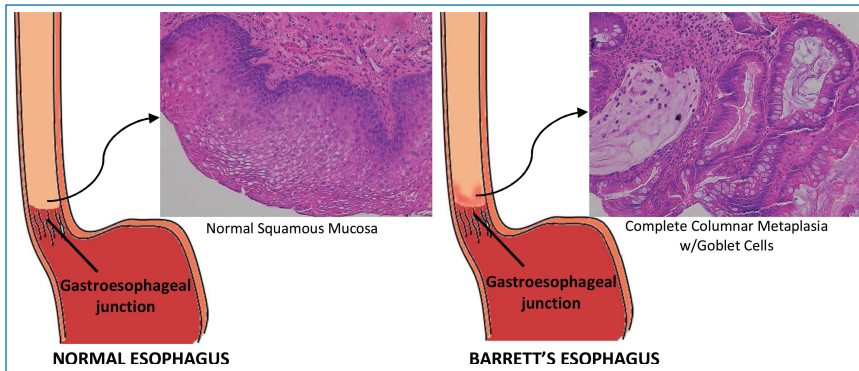


Figure 1. Diagram showing changes in epithelium in the development of Barrett's esophagus. Stratified squamous epithelium of the esophagus undergoes metaplasia to mimic the columnar epithelium with goblet cells found in the intestines. Modified from Spechler.⁷⁴

the most widely studied area in the context of Barrett's esophagus and esophageal adenocarcinoma.

DNA Methylation

One epigenetic mechanism is DNA methylation, where methyl groups are added to gene promoter sequences. This methylation primarily occurs on cytosine bases in cytosine–guanine (CpG) dinucleotides, especially when the cytosine and guanine contents are greater than 50% in the DNA sequence.¹¹ Hypermethylation of these CpG islands on the promoter region results in transcriptional silencing, which decreases the expression of genes, while hypomethylation results in increased expression.¹¹ The enzymes, DNA methyltransferases (DNMTs) catalyze DNA methylation. Changes in these enzymes can lead to aberrant methylation of genes. The most extensively studied DNMT in esophageal cancer is O⁶-methylguanine-DNA methyltransferase (MGMT).¹² MGMT mutations have been implicated in a number of cancers, including esophageal squamous cell carcinoma.¹³ Hypermethylation of this gene has been found to be associated with esophageal adenocarcinoma, but not necessarily with a patient's outcome.^{12,14} The only other DNMT that seems to have been studied in esophageal cancer is DNMT1. Overexpression of the DNMT1 gene was found to be associated with ESCC and correlated with lymph node metastasis.¹⁵ Additional investigation into DNA methyltransferases is indeed a key area to study in esophageal adenocarcinoma.

There are three main mechanisms in which DNA methylation can result in carcinogenesis: base substitution gene mutation, where a 5-methylcytosine is deaminated to thymine; aberrant DNA methylation, which can be associated with allelic loss; and hypermethylation, which may correlate to inactivation of tumor suppressor genes.¹⁶ Hypermethylation-associated silencing of tumor suppressor genes is the most recognized epigenetic disruption, first discovered in the retinoblastoma gene (*Rb1*).¹⁶

In EAC, hypermethylation of genes has been extensively studied. *Table 1* summarizes the results of studies examining hypermethylation in various genes in Barrett's esophagus and esophageal adenocarcinoma. While these studies characterize hypermethylation, they do not discuss the exact functional loss that correlates with different degrees of hypermethylation. Since one of the goals in studying EAC is to establish a biomarker that indicates prognosis of patients, an important aspect that needs to be studied is the differences in methylation found in normal esophageal mucosa compared to levels found in other tissues

and in EAC. It is also important to note that while hypermethylation of these genes correlates with certain stages of EAC, it is not necessarily a causal relationship. In fact, since these studies do not establish a temporal relationship between aberrant methylation patterns and dysplasia, hypermethylation could be a result rather than a cause of the dysplasia. Nonetheless, careful and well-designed studies are warranted to establish causal or consequential effect of hypermethylation of genes at various stages of the initiation, progression and chronicity of metaplasia–dysplasia–adenocarcinoma in the esophagus.

In order to establish these differences, the first step is to differentiate between

methylation patterns in normal squamous esophagus versus patterns in other normal tissues. For example, in one study it was found that *MT3* gene has a large CpG island, and methylation level of the promoter of this gene was high in normal stomach, but low in normal esophagus.¹⁷ This study also found that patients with BE and EAC had hypermethylated *MT3* in the esophagus.¹⁷ On the other hand, some genes are not highly methylated in most tissues. Therefore, it is reasonable to speculate that hypermethylation in any tissue could induce pathological lesions. This could be supported by the study of Kwong et al. who found that high levels of methylation in the *DLEC1* gene were associated with carcinogenesis in the lung, kidney, and esophagus.¹⁸

In addition to looking at normal mucosa, the ideal way to stratify prognosis of patients who develop GERD is to look at methylation patterns in the different stages of dysplasia (*Figure 1*). A study by Agarwal et al. describes the stages of progression as shown below⁶:

Normal Squamous Mucosa → Inflammation (GERD) → BE Metaplasia → Low-Grade Dysplasia → High-Grade Dysplasia → EAC

Most cases of Barrett's esophagus do not progress to esophageal adenocarcinoma. Currently, the best marker of EAC is high-grade dysplasia, but there is no biomarker to predict transition of BE into low or high-grade dysplasia.¹⁹ One study found that Wnt-related genes, such as *APC*, *SFRP1*, and *WIF1*, are more highly methylated in the development of neoplasia from metaplasia, than in the development of metaplasia.⁶ Wnt signaling is present in healthy esophagus, and it has been hypothesized that normal Wnt signaling can result in a change in gene expression.²⁰ Trowbridge et al. proposed that epigenetic changes, including the methylation of *SOX17* promoter may play a role in allowing normal Wnt signaling to result in a change of gene expression.²¹ However, potentially additional mechanisms cannot be ruled out.

Similarly, P16 hypermethylation is seen in higher frequency in Barrett's dysplasia than in the Barrett's metaplasia.²² Another study found that the hypermethylation of *CDKN2A*, *TIMP3*, *ESR1* genes is associated with the onset of Barrett's metaplasia.²³ Furthermore, the study by Wild et al. found that E-cadherin hypermethylation is an important marker in the transition from dysplasia into adenocarcinoma.²⁴ Findings from additional similarly designed studies would help to further characterize the different stages of dysplasia in esophageal adenocarcinoma.²³ While some of these genes are known to be hypermethylated in EAC, their exact role

Classification	Gene	Full name	Function	Citations
Cell cycle control gene	CDKN2A	P16	Cell cycle control	6,44
DNA repair genes	MGMT	O6-methylguanine DNA methyltransferase	DNA repair	29,45–47
Growth factor response related genes	CRBP1	Cellular retinol binding protein 1	Retinol transport	6,8,44
	IGFBP7	Insulin-like growth factor binding protein 7	Modulates binding of insulin-like factors to IGF receptors	48
	SOCS3	Suppressor of Cytokine signaling 3	Suppression of JAK/STAT pathway	1,6,8,46
Metastasis antagonizing genes	CDH1	E-cadherin	Cell adhesion	1,6,40,49,50
	CDH13	T-cadherin	Cell adhesion, proliferation, metastasis	1,6,10–12
Proapoptotic genes	DAPK1	Death associated protein kinase 1	Apoptosis	12,45
	RUNX3	Runt-related transcription factor 1	Proapoptotic factor in TGF- β Pathway	6,8,49,50
Tumor suppressor gene	AKAP12	A-kinase anchoring protein 12	Controls cell signaling, cell adhesion, mitogenesis, and differentiation	6,8,50
<i>Wnt</i> signaling related genes	APC	Adenomatous <i>Polyposis coli</i>	Involved in cell adhesion through its interaction with β -catenin–cadherin proteins.	1,8,29,44,48,50,51
	SFRP1	Secreted frizzled-related protein 1	Antagonist of Wnt protein receptors	6,45,47
	WIF1	Wnt inhibitory factor 1	Wnt-signaling pathway inhibitor	6,8,44,50
Other genes with tumor suppressive functions	CALCA	Calcitonin	Regulates calcitonin levels through adenylate cyclase	6,40
	ESR1	Estrogen receptor α	Hormone receptor in mammary cells	1,6,8,40
	EYA4	Eyes absent homolog 4	Transcriptional activator important for function of the organ of Corti	1,8,50
	GPX3	Glutathione peroxidase	Catalyzes the reduction of hydrogen peroxide	1,4,6,8,44,47
	GSTM2	Glutathione S-transferase Mu 2	Glutathione transferase activity	45,50,52
	MYOD1	Myoblast determination protein 1	Muscle differentiation	1,6,40
	NELL1	Protein kinase C-binding protein NELL1	Cell growth regulation and differentiation	6,44,50
	RPRM	REPRIMO	Regulates p53-mediated cell cycle arrest	6,8,41,50
	SST	Somatostatin	Somatostatin hormone	44,50
	TAC1	Protachykinin-1	Tachykinin peptide hormone	6,44,50
	TIMP3	Tissue inhibitor of metalloproteinase	Metalloproteinase inhibitor	1,6,40,44,47,50,53

Table 1. Genes with hypermethylation patterns reported in the progression of Barrett's esophagus and/or esophageal adenocarcinoma.

is unknown. Also, a few of these genes, for example, *SFRP1*, have been characterized in esophageal squamous cell carcinoma, and may also be hypermethylated in EAC.²⁵ This information may be helpful in developing hypermethylation profiles of various genes. Comparing gene profiles at various stages of the esophageal dysplastic sequence could increase the specificity of the results and help clarify which genes may truly be responsible for inducing phenotypic changes.

In addition to the findings of hypermethylation in several loci of the genome, some studies have observed that the entire genome in cells of BE and EAC is hypomethylated when matched with normal squamous mucosa.²⁶ Global hypomethylation of CpG islands is present to some extent in all cancers, and is observed in EAC as early as low-grade dysplasia.²⁶ While hypomethylation of specific genes has not been extensively studied, there are only a few potential oncogenes that are up-regulated, due to

hypomethylation. These genes include Deleted in Malignant Brain Tumor 1 (*DMBT1*),⁶ *CXCL1*, and *CXCL3* genes,⁶ and *CDX1* and *CDX2*.¹⁰ *CXCL1* and *CXCL3* play a role in spinal cord development by inhibiting the migration of oligodendrocyte precursors.²⁷ *CDX1* and *CDX2* genes are essential for skeletal and intestinal development.²⁸ These genes are usually expressed in the mucosal epithelium from the duodenum to the rectum, but one study demonstrated their presence in EAC tissue.²⁸

Histone Acetylation

Histone acetylation is another epigenetic mechanism that affects chromatin by acetylation of histone proteins. Levels of acetylation/deacetylation of histone proteins are determined by two opposing enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs).²⁹ Hypoacetylation leads to silencing of gene expression, while hyperacetylation leads to

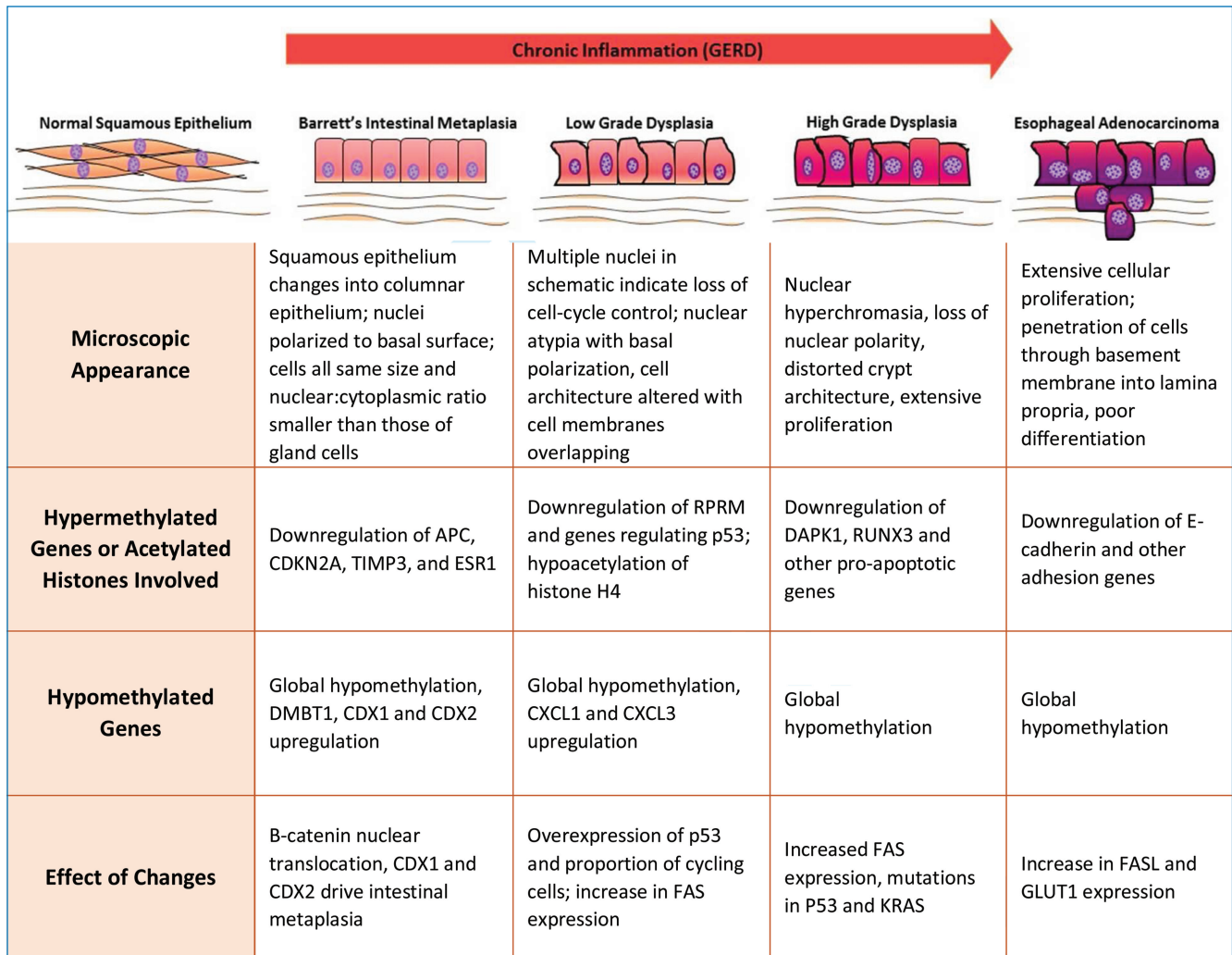


Figure 2. Schematic of role of hypermethylated genes and acetylated histones in the progression from normal mucosa to esophageal adenocarcinoma. Chronic inflammation due to gastroesophageal reflux disease is understood to be the primary factor that drives this pathogenesis. The figure offers a potential role for hypermethylated genes and altered histones in this progression.^{25,75-78}

gene activation.²⁹ There are not many studies examining the role of histone acetylation in the carcinogenesis of esophageal adenocarcinoma.

One study on esophageal squamous cell carcinoma (ESCC) found that histone H4 of esophageal carcinoma cells was significantly hyperacetylated in the early stage of cancer and progressively changed into a hypoacetylated state as the cancer progressed (Figure 2).²⁹ The only other study that focused specifically on histone acetylation theorized that this progress may have an effect on the expression of *INHBA* (activin—a ligand in the TBG-β superfamily) in EAC, but the results were not statistically significant.³⁰ Since there is an overlap between EAC and ESCC, this is a potential important area for future research and additional studies are warranted to dissect the role of histone acetylation in ESCC and EAC.³⁰

MicroRNA

MicroRNAs (miRNA) are small noncoding strands of RNA that are involved in the regulation of transcription and translation by binding to complementary strands of DNA and RNA. They

are smaller and perhaps better biomarkers than mRNA. These molecules play an important role in the modification of gene expression, because while not all genes are targeted by miRNA, one miRNA can target multiple genes, leading to large changes in gene expression.²⁶ Some studies suggest that miRNA may be more helpful than methylation in risk stratification for progression of BE to EAC.³¹ Several miRNAs that have been implicated in EAC (Table 2). This is the first report that summarized the current findings on the type of miRNA involved in various stages of the disease pathogenesis with their expression and the target (Figure 3).

In one study, the expression of four miRNAs (miR-192, miR-194, miR-196a, and miR-196b) was significantly higher in esophageal tissue of patients with progression to esophageal adenocarcinoma than in patients who did not show disease progression.³¹ Another study found that miR-196a could be a potential biomarker in the progression of EAC—its target genes are *SPRR2C*, *S100A9*, and *KRT5* whose expression is characteristically decreased or lost during neoplastic transformation of esophageal tissue.³² In this study, miR-196a was found to be 10- to 100-fold higher in precancerous lesions and EAC than in normal squamous

Stage	MicroRNA	Expression	Target	Citations
NSM to BE	hsa-miR-136*	Increased	AEG-1 and Bcl-2	34,37,54
BE to LGD	miR-21	Increased	PTEN, FASL, TIMP3, and RECK, activating ERK1/2/MAPK pathway	26,31,34,37,43,55–57
	miR-31	Increased	EMP1 (epithelial membrane protein 1), KSR2 (kinase suppressor of ras 2) and RGS4 (regulator of G-protein signaling 4)—found in esophageal squamous cell carcinoma	36,56,58
BE to EAC	miR-25	Increased	Bcl-2-like protein 11 [†] —apoptosis	34,59,60
	miR-93	Increased	CDKN1A—cell cycle	26,60
	miR-106b-25	Increased	CDKN1A—cell cycle	34,36,47,60–62
	hsa-miR-136*	Decreased	‡	34,37
	hsa-miR-192	Increased	DHFR, CDC7, LMNB2, MAD2L1, CUL5—cell cycle, cell proliferation	31,34,36,37,56,60,62
	hsa-miR-194*	Increased	EP300—metastasis	31,36,37,56,60
	miR-196a/b	Increased	ANXA1, SPRR2C, S1009, and KRT5—apoptosis	24,32,36,60–62
	hsa-miR-203	Decreased	ABL1, TP63—cell proliferation	11,26,34,36,37,43,56,60,62,63
	hsa-miR-205	Decreased	HER3, PRKCD, VEGF-A—cell proliferation, EMT	11,26,29,34,36,37,37,43,60,62,63
	hsa-miR-223	Increased	ARTN, a known tumor metastasis-related gene	36,36,37,61,64
	hsa-miR-424*	Increased	‡	9,11,29,34,36,37
	hsa-miR-450a	Increased	‡	34,37
LGD to HGD	miR-15b	Increased	Bcl-2 ⁶²	36
	miR-20b	Decreased	‡	34,36,62
	miR-23b	Decreased	c-Myc	34,36,65
	hsa-miR-101	Increased	rap1GAP—tumor suppressor gene	36,37,65,66
	miR-125b	Increased	CYP24, ERBB2, ERBB3—cell proliferation	11,34,36,43,60
	miR-181a/b	Decreased	TIMP-3 [†]	32,36,55,67
	miR-197	Increased	‡	36
	miR-200a*	Decreased	E-cadherin transcriptional repressors ZEB1 and ZEB2	36,55,57,68
	miR-215	Increased	ZEB2	26,34,56,62,68
	miR-513	Increased	‡	36
	miR-636	Decreased	‡	36
	HGD to EAC	miR-27b	Decreased	ST14, CYP1B1—cell proliferation, cell migration, invasion, drug metabolism
miR-28		Increased	‡	36
miR-30a-5p		Increased	‡	34,36
miR-99a		Decreased	mTOR	11,29,34,36,59,62,69
miR-126		Increased	EGFL7 [†]	36,57,59,70
miR-143		Increased	FSCN1	26,34,36,55,56,59,62
miR-145		Increased	FSCN1	26,34,36,37,56,57,59
miR-149		Decreased	FOXM1 [†] —EMT	36,71
miR-181a/b		Increased	TIMP-3 [†]	32,36,55
miR-193a/b		Decreased	‡	36
miR-199a		Increased	Brm	36,61,72
miR-210		Decreased	MNT [†] —myc antagonist, FGFR1	24,34,36,65,73
miR-345		Decreased	‡	36
miR-494		Decreased	‡	36
miR-513		Decreased	‡	36
miR-617		Decreased	‡	36
let-7a/b/c		Decreased	IL-6, Ras, HMGA2	32,34,36,57,60,62,63,65

Table 2. Continued.

Stage	MicroRNA	Expression	Target	Citations
EAC	miR-16-2	Increased	RAR-b2 [†]	36,57
	miR-30e	Increased	‡	55,57
	miR-34a	Decreased	NF-κB inhibition of c-Met and cyclin D1 protein expression	36,55,57
	miR-195p	Increased	‡	36,55,57
	miR-221	Increased	p27Kip1 and CDX2	31,33
	miR-222	Increased	p27Kip1 and CDX2	31,33
	miR-375	Decreased	PDK1, JAK2	29,31,33,34,36,37,44,55,56,61,65
	hsa-miR-518b	Decreased	‡	36,37,55

The nomenclature of miRNA is included as found in the original studies. Hsa prefix indicates that the miRNA is of human origin; mir refers to an immature transcript, compared to a mature (miR) transcript.¹¹ The number refers to order in which these molecules were discovered (i.e. miR-21 was the 21st miRNA molecule to be discovered).¹¹ A letter after the number indicates an identical sequence found from a different part of the genome.¹¹ Also, since miRNAs are processed from miRNA hairpins, multiple miRNAs may be obtained from a single pretranscript, so 5p or 3p is included to indicate whether the molecule came from the 3' or 5' end.¹¹ Finally, an asterisk indicates that this particular miRNA is found in lower levels than another transcript processed from the same pre-miRNA without an asterisk.¹¹

[†]Potential target.

[‡]Unknown target.

BE = Barrett's esophagus; EAC = esophageal adenocarcinoma; HGD = high-grade dysplasia; LGD = low-grade dysplasia; NSM = normal squamous mucosa.

Table 2. Altered expression of micro RNAs reported in BE and/or EAC.

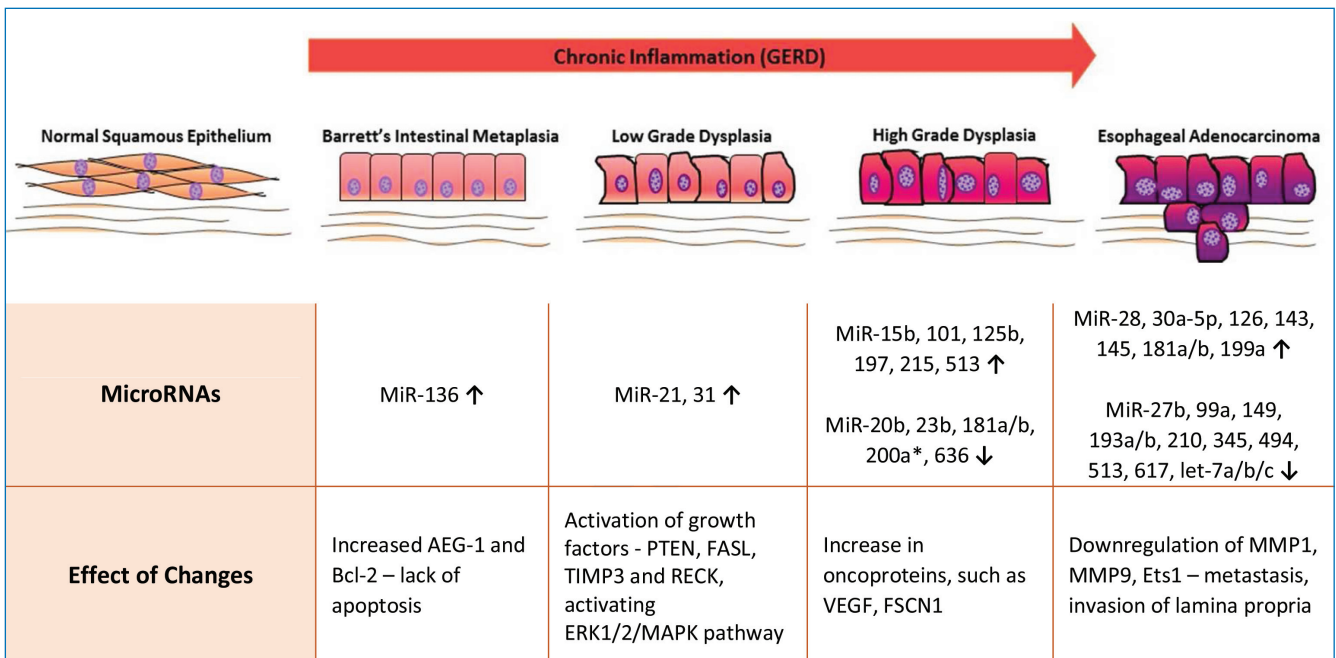


Figure 3. Schematic of role of microRNAs esophageal adenocarcinoma progression. The figure offers a potential role for specific miRNAs in the different stages of pathogenesis.^{76,77,79}

mucosa (NSM), and levels of miR-196a proportionally increase with higher histological grades of dysplasia.³² This is an important point, because all miRNAs do not follow this pattern. For example, miRNA-136, miRNA-181, and miRNA-513 are included twice in this table, because they have been implicated in various stages with different effects. Up-regulation versus down-regulation of these miRNAs seems to have a role in different stages of EAC.

MiRNA-21 is one of the most significant miRNAs, because it has been implicated in the carcinogenesis of other tissues, such as breast cancer and lung cancer.¹¹ MiRNA-21 is important for EAC because it has been found to be up-regulated in a progressive manner through the Barrett's metaplasia–dysplasia–adenocarcinoma sequence, making it a useful biomarker in the

diagnosis and prognosis of EAC. MiR-221 and miR-222 may also be useful, because a couple of studies found that their expression is higher in EAC than in the surrounding BE. Due to GERD, esophageal cells are exposed to bile acids, which may activate farnesoid X receptor and up-regulate levels of miR-221/222.³³ The result of this is a reduction in levels of p27Kip1 and degradation of CDX2.³³ As a result, levels of p27Kip1 and CDX2 were lower in areas of EAC than in those of BE.³¹

While most of the miRNAs that have been examined so far appear to correlate with higher levels in the progression of EAC, some significant miRNAs that may be decreased in BE and EAC are let-7c, miR-203, and miR-205.²⁶ MiR-375 was the only miRNA found to be significantly down-regulated in EAC and unchanged

in BE metaplasia.³⁴ The most comprehensive study available on the up-regulation and down-regulation of miRNAs in BE and EAC is the study by Wu et al.³⁴ The most important biomarkers to examine would be those that are significantly different in EAC versus BE metaplasia. While the study by Wu et al.³⁴ describes many miRNAs, it does not identify the target genes of these molecules. This is an important area that needs to be explored.

Other studies focused on miRNAs that are specifically involved in various stages of EAC. One study found that 3 oncogenic miRNAs, miR-25, miR-93, and miR-106b, are up-regulated in EAC relative to BE, indicating a potential progressive correlation of these miRNAs to the neoplastic process.³⁵ Another study looked specifically at the progression from low-grade dysplasia to high-grade dysplasia, and found that this progression was associated with up-regulation of miR-200a*, miR-513, miR-125b, miR-101, and miR-197, as well as down-regulation of miR-23b, miR-20b, miR-181b, miR-203, miR-193b, and miR-636.³⁶ In the transition from high-grade dysplasia to EAC, miR-126, miR-143, miR-145, miR-181a, miR-181b, miR-199a, miR-28, and miR-30a-5p appear to be up-regulated.³⁶ Let-7a/b/c, miR-193a, miR-345, and miR-494 are down-regulated.³⁶

In addition to the involvement of miRNAs at various stages, miRNA could serve as a prognostic marker. A study by Huang et al.³⁶ found a correlation between miR-126 expression and tumor cell differentiation and lymph node metastasis (LNM), and that miR-16-2 and miR-195p are associated with LNM and higher tumor stage.³⁶ Huang et al. suggest that miRNAs could potentially be good targets for therapy, because most miRNAs exert their effects on multiple target genes. However, since a single miRNA could target many genes, such as those reported by Wang et al.,³⁷ where a single miRNA targeted up to 50 genes, there is a possibility that up-regulation of one gene is regulated by down-regulation of other genes. This could result in globally adverse and unpredictable effects where the role of a specific miRNA in the pathogenesis of esophageal carcinoma would remain unclear.

Long Noncoding RNA

Long noncoding RNA (lncRNA) is similar to miRNA and snoRNA in that these are noncoding strands of RNA. They are usually longer than miRNA (longer than 200 bp). Also, unlike other noncoding RNA molecules, such as miRNA and snoRNAs, lncRNA molecules are not strongly conserved across diverse species.³⁸ While this lack of conservation has been cited as a reason for nonfunctionality, it could also be an evidence to support that noncoding RNAs are subject to different selection pressures. This is a far less studied area than DNA methylation and miRNA, but this could also be a potential epigenetic mechanism.

One published study on lncRNA by Wu et al.³⁹ found a role of lncRNA in the development of BE. These investigators selected AFAP1-AS1 as the lncRNA to study because it was significantly and aberrantly hypomethylated in BE.³⁹ AFAP1 modulates actin filament integrity and serves as an adaptor protein linking Src family members and other signaling proteins to actin filaments.³⁹ AFAP1 plays a role in breast cancer, because it is required for actin stress fiber formation and cell adhesion in breast cancer cells.³⁹ While this was the only study that looked at lncRNA, this field is wide open to explore in regards to epigenetic mechanisms involved in the pathogenesis of esophageal adenocarcinoma.

Expert Commentary and 5-Year Review

One goal in studying epigenetics is to develop risk stratification that is helpful in delivering a clearer prediction of developing

EAC in patients with BE. DNA hypermethylation studies are most helpful in this regard, because different genes have been found to be hypermethylated in different stages of the BE to EAC progression.⁴⁰ One study found that 19 CpG islands segregate into six classes of genes silenced in cancer. Each class undergoes unique epigenetic changes at different steps of disease progression to EAC. The classes were defined as sharing similar epigenetic behaviors. Now that these changes have been characterized, the next step in studying these patterns will be to identify the molecular mechanisms and factors affecting the various CpG island clusters.⁴⁰

In studying epigenetics and its role in esophageal adenocarcinoma, we encounter a number of obstacles. The clinical application of methylated DNA biomarkers for both diagnosis and prognosis of BE and esophageal cancer is limited because there are not enough clinical trials for validation (such as phase 2–3 biomarker studies).⁸ Also, many of the studies that have been done involve the characterization of expression without any link to function. Prospective studies would be helpful to achieve this goal. There is a need for longitudinal studies between patients with nonprogressive BE and patients with progressive BE. However, these studies could be challenging to complete due to the amount of time and resources that are required.

In addition, significant studies have been done on the hypermethylation and miRNA expression, but more focus on functional studies of the gene is required to see exactly what effect miRNAs have.²⁶ Chronic inflammation in the esophagus clearly has an effect on the progression of this cancer, so studying the association between inflammatory processes and inflammatory alterations in miRNA expression is a critical area of research.²⁶ This is difficult to achieve since miRNA molecules often exert effects on multiple genes.

Since the goal of these studies is to eventually find a useful biomarker, it is important to characterize markers that are as noninvasive as possible. Circulating biomarkers would be less invasive and less expensive. However, the problem with looking at these biomarkers is that the plasma levels might show different methylation patterns based on the severity of the progression. A study by Shah et al. found that the *APC* gene in the plasma was hypermethylated in late-stage EAC, but not in BE; tissue *APC* gene showed hypermethylation even in early stages.⁴¹ Thus, plasma levels may lag tissue levels in showing hypermethylation patterns, making them less than ideal despite being less invasive. The use of biopsy tissue would be best for such studies. However, this could involve a more invasive procedure for the patient, if additional biopsy tissue is collected.

In regard to hypermethylation, most of the published studies focus on the role of tumor suppressor genes in the development of BE and EAC, but few studies examined the inflammatory processes in the development of BE. Damage from GERD leads to increased activity of COX-2, suggesting a potentially important role in the methylation of genes regulating inflammation.⁴² This may be an important area for future investigation, because it could yield more helpful findings on the molecular mechanisms leading to dysplasia. These biomarkers could also be more useful in earlier stages of the disease. Most of the studies that show some sort of risk stratification do not discuss the progression of BE into low-grade dysplasia and further progression into higher grades of dysplasia. These inflammatory mechanisms may hold the key to finding the likelihood of cancer at much earlier stages.

While there are a number of studies focusing on the up-regulation and down-regulation of miRNAs in EAC and BE, there are limited studies that aim to determine the target genes of these miRNA molecules. The problem with determining target genes is that there are too many targets to be able to realistically pinpoint the cellular mechanism that leads to neoplasia. For example, a study by Wang et al. found the two most significant miRNA markers where miR-21 was up-regulated and miR-203 was down-regulated.³⁷ MiRNA profiles of BE and EAC are more similar to each other than to the profiles of any squamous epithelial tissue. Perhaps this makes sense, since EAC arises from columnar epithelium, rather than squamous epithelium.⁴³

Although miRNAs do have the potential to be useful biomarkers, with the ease of accessibility, their function is not fully understood since current studies have only characterized expression levels. It is important to perform more studies that examine the target genes of these molecules. However, this is a challenging goal, since some miRNAs have over 100 target genes. This is critical from a treatment standpoint, because altering the levels of miRNAs can have large global effects. At the same time, the large number of target genes poses a problem in identifying specific genes that are affected.

Conclusion

Overall, significant research has been done in hypermethylation, and these studies may show the best biomarkers for the future, but prospective studies would provide stronger evidence for correlation. Histone acetylation seems to play an important role in esophageal squamous cell carcinoma, and may show good results in EAC. Studies on miRNAs are promising, but more work needs to be done on the effect of gene function. While presence of, or lack thereof, many miRNA molecules has been found, but there is no link to the function of these miRNAs. Studies on lncRNA are very limited, and there is not enough information to support whether lncRNA will be a useful biomarker to predict the development of EAC. An interesting area for future directions would be to examine the relative levels of hypermethylation and miRNA and compare these to the response of the patients to neoadjuvant therapy—if there is a difference in these levels, the method of neoadjuvant therapy may need to be accordingly adjusted.

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Disclosure of Potential Conflicts of Interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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