Risk factors and molecular mechanisms of esophageal cancer: differences between the histologic subtypes

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ABSTRACT

The two major histologic subtypes of esophageal cancer have different risk factors as well as different molecular mechanisms. In this review, the differences in risk factors and genetic/epigenetic alterations between esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) will be discussed. Cigarette smoking and alcohol consumption are risk factors for ESCC, while gastroesophageal reflux, cigarette smoking, and obesity are the main EAC risk factors. Commonly mutated genes of both subtypes are \textit{TP53} and \textit{PIK3CA}. Recent genome-wide analysis revealed that the activation of the RAC1 pathway may contribute to EAC tumorigenesis. Clustered abnormality in copy number was observed in several genes in ESCC, whereas a few genes were specifically altered at high frequency in EAC. Epigenetic changes, such as DNA methylation, histone modifications, and altered expression of microRNAs, have been revealed to influence carcinogenesis and progression of both ESCC and EAC.

Key words: Epigenetic alterations, esophageal cancer, genetic alterations, risk factors

Introduction

Esophageal cancer affects more than 450,000 people every year worldwide\cite{1} and is the 6th leading cause of cancer-related mortality.\cite{2} The two major histologic subtypes of esophageal cancer are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC). ESCCs are by far more common in South East and Central Asia (79\% of the total global ESCC cases), while the highest number of EAC is found in Northern and Western Europe, North America and Oceania (46\% of the total global AC cases).\cite{3} The remarkable variations in geographic distribution indicate that different environmental risk factors likely affect the occurrence of esophageal cancer.

Recent progress in molecular biology has revealed that several genetic and epigenetic alterations are implicated in both carcinogenesis and progression of esophageal cancer. Genetic alterations include a chromosomal loss or gain, loss of heterozygosity (LOH), and amplification or mutations of genes. Epigenetic changes, such as DNA methylation, histone modifications, and altered expression of microRNAs regulate gene expression through mechanisms other than changes in DNA sequence. It has become evident that molecular mechanisms also differ greatly between the two histologic subtypes.

In this review, the differences in both risk factors and molecular mechanisms between ESCC and EAC will be summarized.

Risk Factors

There are different risk factors between ESCC and EAC. Demonstrated in Table 1 are the major risk factors for each histologic subtype.

Both cigarette smoking and alcohol consumption are well-established risk factors for ESCC,\cite{4,5} with the risk in heavy smokers/drinkers being 50 times greater than those who neither drank nor smoked.\cite{6} Recently, deficiency in the enzyme aldehyde dehydrogenase 2 (ALDH2), which causes so-called alcohol flushing response, has been revealed to increase the risk of alcohol-related ESCC.\cite{7} In East Asian populations, there is a variant of ALDH2, resulting from the replacement of glutamate at position 487 with lysine, with the lysine allele encoding an inactive protein.\cite{8} Drinking hot beverages may also increase the risk of ESCC.\cite{9} In addition, patients with achalasia are at markedly increased risk of developing ESCC,\cite{10} while both ESCC and EAC may develop as a late complication of caustic injury.\cite{11} Oncogenic human papillomaviruses may increase the risk of ESCC, but the evidence is inconclusive.\cite{12}

Gastroesophageal reflux disease (GERD), cigarette smoking, and obesity are the main EAC risk factors.\cite{13} At least weekly symptoms of GERD increases the odds
of EAC five-fold, while daily symptoms increased the odds seven-fold, when compared with those with less frequent episodes.\(^1\) The relative risk of esophageal and gastric cardia AC was 2.32 for current smokers and 1.62 for ex-smokers, as compared with never-smokers.\(^2\) However, a meta-analysis provided definite evidence of an absence of association between alcohol drinking and esophageal and gastric cardia AC risk.\(^3\) A systematic review and meta-analysis revealed a high body mass index (BMI) to be associated with a summary odds ratio for gastroesophageal AC of 1.5.\(^4\) A recent prospective cohort study in the United States found that a BMI ≥ 35 was associated with a hazard ratio of 3.67 compared with those with a normal-range BMI.\(^5\) Obesity may predispose to reflux through mechanical means, while adipokines and cytokines secreted from adipocytes and inflammatory cells are known to influence tumor development.\(^6\) Helicobacter pylori infection has been reported to actually decrease the risk of EAC by 41%\(^7\) through gastric atrophy, which leads to acid reduction.

Radiotherapy for thoracic diseases, such as breast cancer and Hodgkin’s lymphoma, increases the risk of both ESCC and EAC.\(^8\) The incidence of both ESCC and EAC increases with age. There is a strong male predominance with up to eight men/one woman for EAC and three men/one woman for ESCC.\(^9\) Fat distribution in obese men is predominantly abdominal, and increasing abdominal diameter has been associated with an increased EAC risk.\(^10\) However, the male predominance of ESCC can be explained by the prevalence of smoking and alcohol drinking among males.\(^11\) Although an inhibitory effect of estrogen in the growth of esophageal cancer cells has been reported, there is no firm conclusion on the role of estrogen in human esophageal cancer etiology.\(^12\) The familial form of ESCC is rare, although familial aggregation has been reported in a high incidence area in China.\(^13\) In contrast, familial clustering of Barrett’s esophagus and EAC has been observed. In a European cohort study, 7% of cases of Barrett’s esophagus and EAC were familial.\(^14\)

The efficacy of endoscopic surveillance for high-risk individuals is controversial. Both lugol chromoendoscopy and an innovative optical image-enhanced technology such as the narrow band imaging have been reported to be useful in detecting early ESCC.\(^15\) In addition, endoscopic esophageal surveillance has been recommended for newly-diagnosed head and neck cancer patients.\(^16\) However, there is no study evaluating the efficacy of endoscopic surveillance or screening among people heavily exposed to ESCC risk factors. In contrast, endoscopic screening is recommended for patients with multiple risk factors in Barrett’s esophagus, although there is no randomized clinical trial that has shown efficacy in preventing deaths due to esophageal cancer.\(^17\) For patients with Barrett’s esophagus without dysplasia, endoscopic surveillance at intervals of 3-5 years has been recommended, and endoscopic eradication therapy is the treatment of choice for those with high-grade dysplasia (HGD).\(^18\) Recently, however, lengthening surveillance or discontinuing surveillance of patients with persistent non-dysplastic Barrett’s esophagus (NDBE) has been discussed because of an annual cancer incidence of only 0.1-0.3% in such patients.\(^19\)

### Molecular Mechanisms

#### Mutations

Recently, the results of whole-exome or whole-genome sequencing to identify somatic mutations in ESCC\(^20\) and EAC\(^21\) have been reported. The frequently mutated genes in esophageal cancers are shown in Table 2. The commonly mutated genes of both subtypes are TP53 and PIK3CA. TP53 is a major tumor-suppressor gene, its primary function being maintenance of genetic stability and DNA repair capacity.\(^22\) PIK3CA is a kinase activator of the phosphoinositide 3-kinase (PI3K)/AKT pathway and is frequently mutated in many types of human cancers, including ESCC.\(^23\) NOTCH1, FAT1, FAT2, KMT2D and ZNF750 are also significantly mutated in ESSC. NOTCH1 encodes one of the notch family receptors, and the notch signaling is a key pathway of the stem cell signaling network.\(^24\) There are other recently identified mutated genes\(^25\) and the much about the functions remains to be researched.

#### Table 1: Risk factors of esophageal cancer

<table>
<thead>
<tr>
<th>Squamous cell carcinoma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cigarette smoking</td>
<td>Gastro-esophageal reflux disease</td>
</tr>
<tr>
<td>Alcohol drinking</td>
<td>Barrett’s esophagus</td>
</tr>
<tr>
<td>ALDH2 deficiency</td>
<td>Reflux symptoms</td>
</tr>
<tr>
<td>Drinking very hot liquids</td>
<td>Obesity</td>
</tr>
<tr>
<td>Achalasia</td>
<td>Cigarette smoking</td>
</tr>
<tr>
<td>Caustic injury</td>
<td>Diet (high in processed meat, low in fruits, vegetables)</td>
</tr>
<tr>
<td>History of thoracic radiation</td>
<td>History of thoracic radiation</td>
</tr>
<tr>
<td>Tylosis</td>
<td>Anticholinergic agents</td>
</tr>
<tr>
<td>Human papilloma virus infection</td>
<td>Family history</td>
</tr>
<tr>
<td>N-nitrosamines</td>
<td>Helicobacter pylori infection (decreased risk)</td>
</tr>
</tbody>
</table>

#### Table 2: Representative mutated genes in esophageal cancer

<table>
<thead>
<tr>
<th>Squamous cell carcinoma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>TP53</td>
</tr>
<tr>
<td>KMT2D</td>
<td>CDKN2A</td>
</tr>
<tr>
<td>FAT1</td>
<td>SMAD4</td>
</tr>
<tr>
<td>FAT2</td>
<td>ARID1A</td>
</tr>
<tr>
<td>NOTCH1</td>
<td>PIK3CA</td>
</tr>
<tr>
<td>ZNF750</td>
<td>SPG20</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>TLR4</td>
</tr>
<tr>
<td></td>
<td>ELMO1</td>
</tr>
<tr>
<td></td>
<td>DOCK2</td>
</tr>
</tbody>
</table>

Bold: Genes commonly mutated in both subtypes
CDKN2A, SMAD4, ARID1A, SPG20, TLR4, ELMO1, and DOCK2 are significantly mutated in EAC. p16\textsuperscript{INK4a}, encoded by CDKN2A, inhibits CDK4 and 6 that bind to cyclin D1 and blocks abnormal cell growth and proliferation.\textsuperscript{[41]} SMAD4 is a key intracellular mediator of transforming growth factor-beta signaling and is known to act as a tumor suppressor.\textsuperscript{[42]} ARID1A, which is one of the chromatin remodeling genes, is frequently mutated in a variety of human cancers.\textsuperscript{[43]} Among the remaining four newly identified genes, ELMO1 and DOCK2 are upstream modulators of RAC1 GTPase, suggesting the potential activation of the RAC1 pathway as a contributor to EAC tumorigenesis.\textsuperscript{[56]}

Recently, comparison of mutated genes among NDBE, HGD, and EAC revealed the majority of recurrently mutated genes in EAC, except TP53 and SMAD4, were also mutated in NDBE.\textsuperscript{[44]} Mutations of TP53 and SMAD4 were stage-specific, confined to HGD and EAC, respectively.\textsuperscript{[44]}

**DNA copy number alterations**

Clustered abnormality in copy number was observed in several genes in ESCC [Table 3], whereas a few genes were specifically altered at high frequency in EAC.\textsuperscript{[45]} Instead, EAC samples demonstrated more widespread genomic instability and the total DNA copy number alterations were an independent prognostic factor.\textsuperscript{[45]}

Amplification and LOH observed in ESCC are summarized in Table 3. Amplification and overexpression of CCND1, which positively regulates G1/S transition, are frequently observed.\textsuperscript{[46]} The PI3K/AKT pathway is activated by amplification and overexpression of receptor tyrosine kinases (RAS-association domain family 1 (RASSF1), MGMT, MLH1, and MSH2, causes decreased expression of these genes and has been known to affect carcinogenesis of ESCC.\textsuperscript{[54]} [Table 4]. E-cadherin, encoded by CDH1, is a calcium-dependent adhesion molecule that plays a crucial role in the maintenance of intercellular junctions in normal epithelial cells.\textsuperscript{[55]} The RARB gene encodes retinoic acid receptor beta, a central regulator to normal growth and differentiation of a variety of epithelial cells.\textsuperscript{[56]} The RASSF1 encodes a protein similar to RAS effector proteins. RASSF1A protein modulates a broad range of cellular functions essential for normal growth control.\textsuperscript{[57]} The MGMT gene encodes O\textsuperscript{6}-methyl-guanine-DNA methyltransferase, a DNA repair protein.

**Table 3:** Representative amplified or deleted genes in squamous cell carcinoma of the esophagus

<table>
<thead>
<tr>
<th>Genes</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCND1</td>
<td>11q13</td>
<td>Cell cycle progression</td>
</tr>
<tr>
<td>FGFR1</td>
<td>8p11</td>
<td>Mitogenesis, differentiation</td>
</tr>
<tr>
<td>EGFR</td>
<td>7p12</td>
<td>Proliferation</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>3q26</td>
<td>Cell growth, survival, proliferation</td>
</tr>
<tr>
<td>MYC</td>
<td>8q24</td>
<td>Cell cycle progression, transformation</td>
</tr>
<tr>
<td>SOX2</td>
<td>3q26</td>
<td>Stemness</td>
</tr>
<tr>
<td>KRAS</td>
<td>12p12</td>
<td>Proliferation</td>
</tr>
</tbody>
</table>

**Table 4:** Representative hypermethylated genes in esophageal cancer

<table>
<thead>
<tr>
<th>Squamous cell carcinoma</th>
<th>Adenocarcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>APC</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>TIMP3</td>
</tr>
<tr>
<td>CDH1</td>
<td>CDKN2A</td>
</tr>
<tr>
<td>FHIT</td>
<td>CDH1</td>
</tr>
<tr>
<td>RARB</td>
<td>MGMT</td>
</tr>
<tr>
<td>RASSF1</td>
<td>DAPK</td>
</tr>
<tr>
<td>MGMT</td>
<td>FHIT</td>
</tr>
<tr>
<td>MLH1</td>
<td>AKAP12</td>
</tr>
<tr>
<td>MSH2</td>
<td>SOCS-3</td>
</tr>
</tbody>
</table>

Bold: Genes commonly hypermethylated in both subtypes
enzyme, which removes methyl- or alkyl-groups from guanidine after chemical modulation, therefore protecting cells from G to A mutations.\[58\] MLH1 and MSH2 are two key DNA mismatch repair genes and epigenetic silencing of these genes may lead to microsatellite instability.\[59\]

Promoters of APC, tissue inhibitor of metalloproteinases 3 (TIMP3),\[60\] CDKN2A, CDH1, MGMT, DAPK, FHIT,\[61\] AKAP12,\[62\] and suppressors of cytokine signaling (SOCS)\[63\] have been reported to be frequently hypermethylated in EAC (Table 4). TIMP3 belongs to a family of genes that inhibit matrix metalloproteinases, a group of peptides involved in degeneration of extracellular matrix.\[64\] Death-associated protein kinase 1 is a positive mediator of gamma-interferon-induced programmed cell death.\[65\] A-kinase anchoring protein 12 is a multivalent anchoring protein and an important regulator of the beta2-adrenergic receptor complex.\[66\] SOCS proteins act as negative regulators of JAK/STAT pathways and may represent tumor suppressors.\[66\] Promoter methylation and subsequent transcript down-regulation of SOCS-3 and to a much lesser extent, SOCS-1 were involved in the multistep carcinogenesis of Barrett’s AC.\[67\]

Genome-wide DNA hypomethylation may also contribute to tumorigenesis. Long interspersed element 1 (LINE-1) is a retrotransposon comprising about 17% of the human genome, and the levels of LINE-1 methylation can be a surrogate marker of genome-wide DNA methylation.\[54\] Hypomethylation levels of LINE-1 are frequently observed in ESCC and correlate with a poor prognosis.\[67\] On the other hand, genome-wide methylation analysis also revealed that overall methylation of CpG islands was higher, but outside of CpG islands was lower, in Barrett’s esophagus and EAC tissues than in normal esophageal tissues.\[68\]

Histone modifications, including acetylation, methylation, phosphorylation, and ubiquitination, regulate gene expression and are implicated in carcinogenesis. Levels of acetylation/deacetylation of histone proteins are determined by two opposing groups of enzymes, histone acetyltransferases, and histone deacetylases (HDACs).\[69\] HDAC inhibitors have demonstrated antitumor effects in various cancers.\[70\] Of interest, high HDAC2 expression has been associated with aggressive EAC behavior.\[71\]

MicroRNAs (miRs), small, noncoding RNA molecules consisting of 19-25 nucleotides, also regulate gene expression epigenetically.\[72\] MicroRNAs can act as tumor promoters (onco-miR) through targeting expression of tumor suppressor genes or as tumor suppressors (ts-miR) through targeting expression of oncogenes. miR-21 functions as an onco-miR because it is overexpressed in many types of cancers, including ESCC\[73,74\] and EAC.\[75\] Targets of miR-21 have been shown to be PDCD4 (programmed cell death 4)\[73\] and phosphatase and tensin homolog.\[76\] Serum or serum exosomal miR-21 has been reported to be a biomarker in ESCC.\[77,78\] miR-375 is considered as ts-miR in several cancers, including both histologic subtypes of esophageal cancer.\[79,80\] Reduced levels of miR-375 in cancerous tissue of EAC patients with Barrett’s were strongly associated with a worse prognosis.\[80\] miR-205 was down-regulated in both ESCC and EAC.\[81,82\] Knockdown of miR-205 expression enhanced expression of zinc finger E-box homeobox 2, accompanied by a reduction of E-cadherin, leading to epithelial-mesenchymal transition.\[82\] miR-223 expression was significantly higher in ESCC with an inverse relationship with F-box and WD repeat domain-containing 7, a cell cycle regulatory gene whose protein product ubiquitinates cell cycle regulators such as c-Myc, cyclin E and c-jun.\[83\]

Recently, changes in expression of several miRs have been reported in Barrett’s esophagus.\[84\] miR expressions were compared between 2 groups of patients with Barrett’s esophagus who either developed or did not develop EAC over a course of 5 years.\[85\] As a result, 4 miRs (miR-192, miR-194, miR-196a, and miR-196b) were found to show significantly higher expression in patients with progression to EAC than in those without.

**Conclusion**

In this review, the risk factors and molecular mechanisms of esophageal cancer, with special reference to the differences between two histologic subtypes, have been discussed. In spite of advances in the diagnostic tools and therapeutic strategies, esophageal cancer still remains one of the most lethal malignancies. In order to improve outcomes, early detection of tumors based on knowledge of risk factors is needed. In addition, efforts to identify novel therapeutic targets through molecular biological techniques are essential.

**References**


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