Overview of genetic and epigenetic alterations in the pathogenesis of esophagogastric junctional adenocarcinoma and esophageal adenocarcinoma: recent findings by next generation sequencing

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A B S T R A C T

Esophagogastric junctional adenocarcinoma is commonly treated as esophageal adenocarcinoma (EAC) and has dramatically increased in Western countries for several decades. The similar trend has been observed in Asian countries (not in China). Barrett's esophagus (BE) is a widely accepted precursor of EAC. Recent advances of next-generation sequencing could provide researchers with a better understanding of genetic and epigenetic alterations in the carcinogenesis of EAC. In this review, we have summarized the recently reported major genetic and epigenetic alterations in both BE and EAC. Sonic hedgehog/bone morphogenetic protein axis, which is a key signaling for esophageal development, plays an important role in BE intestinal metaplasia. Single nucleotide polymorphisms related to esophageal organogenesis, such as *FOXF1* and *FOXP3,* are frequently detected in BE patients. During the progression of BE to adenocarcinoma, lacking of normal function of TP53 and CDKN2A by loss of heterozygosity (LOH), mutation, or promoter methylation has been frequently observed. LOH at 9p (coding *CDKN2*A) is an earlier event to EAC carcinogenesis compared to that at 17q (coding *TP53*) LOH. In order to further elucidate the pathogenesis of BE and EAC, it will be necessary to analyze these genetic/epigenetic alterations in combination with clinical data in a large-scale cohort.

Key words: Barrett's esophagus, carcinogenesis, epigenetic, esophageal adenocarcinoma, esophagogastric junctional adenocarcinoma, genetic, intestinal metaplasia

Introduction

Esophagogastric junctional (EGJ) adenocarcinoma is classified as I to III, based on the location of the tumor center or tumor mass, by Rudiger Siewert *et al.*[1] EGJ cancer is considered to be an esophageal cancer, according to the 7th edition of Union for International Cancer Control tumour, node, metastasis classification.[2] EGJ adenocarcinoma/esophageal adenocarcinoma (EAC) has dramatically increased by 600%, mainly in Western countries, over the past few decades, although the current incidence rate shows only a moderate increase.[3] Currently, a similar trend was reported in Asian country.[4] EGJ adenocarcinoma often presents at a late stages despite recent improvements in diagnostic technology and multidisciplinary treatment. The 5-year survival rate is reported to be about 20% and median survival less than one year.[3,5]

Barrett's esophagus (BE) is a widely accepted precursor of EGJ adenocarcinoma/EAC, although the reported risk is around 0.5% per year.[6] Epidemiological studies have revealed that adenocarcinomas occur from BE through multistep morphological changes, such as low-grade to high-grade dysplasia.[6,7] BE and EGJ adenocarcinoma/EAC share poly-genetic/epigenetic alterations.[8] BE can be described as mucosal replacement of normal squamous epithelium with metaplastic columnar mucosa, known as specialized columnar metaplasia, in response to chronic gastroesophageal reflux disease (GERD).[9] Understanding the pathogenesis of BE and EGJ adenocarcinoma/EAC is important in prevention and thus the development of molecular targeting therapy. Here, we review the pathogenesis of EGJ adenocarcinoma/EAC, including BE, focusing on molecular alterations. We use the term EAC and include EGJ adenocarcinoma.

Barrett's Esophagus

BE is defined by American Gastroenterological Association as "BE is the condition in which any extent of metaplastic columnar epithelium that predisposes to cancer development replaces the stratified squamous epithelium that normally lines the distal esophagus."[10] This means a specialized columnar epithelium characterized by columnar cells, goblet cells, and a villous-like structure.[11,12] However, another classification includes two types of BE. One is "junctional or cardiac type," consisting of the predominantly foveolar surface containing mucous glands and resembling cardiac mucous glands. Another one is "gastric-fundic type," containing both parietal and chief cells with atrophic fundic glands.[11-13] Thus, the histological definition of BE remains controversial.

The cell of origin of BE has not yet been elucidated. Six cell types are currently considered as potential origins, including transdifferentiation of esophageal squamous cells,[14] gastric cardia cells,[15] esophageal submucosal gland cells,[16] esophageal progenitor cells,[17] circulating bone marrow cells,[18] and residual embryonic cells at squamo-columunar junction (SCJ).[19]

There are some reports suggesting an association between p63 and intestinal metaplasia. *p63* null embryos have idiopathic metaplasia in SCJ.[20] It has been shown that genetic alterations in metaplastic cells in mice lacking p63 were similar to those in human BE.[21] It has also been suggested that epithelium with such genetic changes may originally exist at SCJ. Also, lack of SRY (sex determining region Y) box 2 (SOX2) induces columnar changes in esophageal epithelium in mice models.[22] Both p63 and SOX2 are essential for squamous epithelial formation during organogenesis. Although these findings were based on studies using rodent esophagus, there are structural differences in the esophageal between rodents and human. For example, in rodents, the esophagus lacks submucosal glands and SCJ is located in mid-stomach. Therefore, findings in rodent models may not be applicable to human BE.

Molecular and Genetic Alterations Related to Intestinal Metaplasia and Intestinal Differentiation

Sonic hedgehog (SHH)/bone morphogenetic protein (BMP) signaling plays an important role in the development of columnar metaplasia, being associated with organogenesis, especially of the esophageal. These are critical molecules for separating trachea from the esophagus[23] and are involved in the development of cell-renewable epithelium.[24] Expressions of SHH and BMP4 are usually low in human squamous epithelia. In BE tissue, however, SHH/BMP4 signaling induces SRY (sex determining region Y) box 9 (SOX9).[25,26] SOX9 subsequently induces CDX2 and MUC2 expression,

which are related to an intestinal phenotype.[27] Furthermore, BMP4 shifts the gene expression profile of normal squamous cells into columnar cells. Because cytokeratin (CK) is a major cytoskeleton molecule, it can be regarded as a representative phenotype of certain cells. CK 13/14 expressions are highly expressed in squamous cells, whereas CK 7, 8, 18, and 20 expressions elevated in BE epithelium.[28] It has been shown that expression of SOX9, but not CDX2 or BMP4, induces squamous epithelial cells formation toward columnar-like epithelium with expression of CK 8.[29] SHH/BMP signaling were also activated in a mouse model with interleukin-1β overexpression. After one year of continuous inflammation, intestinal metaplasia occurred at the SCJ, and the gene expression pattern of those metaplastic cells was similar to those in human BE.[30]

Recent advances of next-generation sequencing have provided the opportunity to elucidate genetic alterations such as single nucleotide polymorphisms (SNPs). The association between SNPs and BE has been clarified. It has been reported that chromosomes 2p24 (rs3072), 12q24 (rs2701108), 6p21 (rs9257809), and 16q24 (rs9936833) are related to risk of BE development.[31,32] Among these SNPs, rs9936833 at 16q24 is located close to *FOXF1*, which is a transcription factor in the SHH signaling pathway. Interestingly, *FOXF1* is associated with embryonic development of gastrointestinal tract formation, especially the esophagus.[33] Also, the importance of *FOXP3*, at 3p14 (rs2687201), which is also known to possess a role in esophageal organogenesis, is based on analyzing datasets of BE or EAC cases.[34] 19p13 (rs10419226) and 9p22 (rs11789015), with significant relation to BE and EAC, has also been identified. rs10419226 SNPs at 19p13 are known as an intronic variant of cAMP-regulated transcriptional co-activators (CRTC1). CRTC signaling exerts oncogenic activities when activated by loss of LKB1 through transcriptional activation of LYPD3, which contributes to esophageal tumor progression.[35] rs11789015 SNP at 9p22 is located at the intron region of *BARX1*. BARX1 is a transcription factor involved in tracheal and foregut organogenesis in developing mouse embryos.[36,37] These findings suggest that key molecules in BE development may overlap with those in esophageal development.

Wnt/β-catenin, and Notch are critical signaling for intestinal differentiation. Wnt family is one of the fundamental mechanisms of cell proliferation, polarity, and differentiation.[38] Wnt signaling pathways include Wnt/β-catenin canonical pathway and Wnt/calcium or Wnt/planar cell polarity non-canonical pathway. Among these, Wnt/β-catenin pathway is associated with intestinal type gene expressions.[39,40] Wnt signaling also regulates *CDX* gene expression, which controls intestinal differentiation, and homeostasis.[41] Notch signaling

also plays an important role in intestinal differentiation in cell proliferation, apoptosis, and normal cell differentiation.[42,43]

SHH, BMP4, SOX9, and CDX2 are key molecules for the development of intestinal metaplasia. SHH/BMP4 axis, which is a key signaling for esophageal development, plays an important role in the intestinal metaplasia of BE. In addition, SNPs that are related to esophageal organogenesis, such as *FOXF1* and *FOXP3,* are frequently observed in BE patients [Table 1].

Genetic Alterations in Progression of BE to EAC

Few cases of BE will develop high-grade dysplasia or adenocarcinoma. The widely accepted molecular events during progression of BE to adenocarcinoma are loss of normal TP53 and CDKN2A function. Mechanisms underlying this have been explained by loss of heterozygosity (LOH), mutation, or promoter methylation. Tumor suppressor genes, *TP53* and *CDKN2A*, are located at 17p and 9p, respectively.[44] 17p LOH occurs frequently in EAC,[45-47] while *TP53* mutation possesses malignant transformation potential during EAC carcinogenesis.[48] 9p LOH has been reported to be the important factor driving to EAC.[44] Somatic mutation of *CDKN2A* has also been detected in EAC cases.[49] In addition, tumors harboring promoter methylation in *CDKN2A* showed a higher risk of EAC progression.[50,51] Although 9p LOH is an earlier event during EAC carcinogenesis compared to 17q LOH, patients with BE harboring 9p LOH experienced much higher incidence of EAC compared to those with 17q LOH.[44]

Comprehensive genetic analysis has provided new insights in the genetic landscape of BE-to-EAC. One group has shown that most mutations in EAC had already occurred in matched BE, using comprehensive genetic analysis on 11 cases with EAC and 2 of BE. Another group analyzed the mutations in selected 26 genes and reported that around half of the cases with BE without dysplasia already possessed mutations. Also, there was no significant difference in frequencies of those mutations between BE without dysplasia, BE with high-grade dysplasia, and EAC.[52] Of note, they also examined associations between frequencies of mutations in the 26 genes and disease stage. They also found that only *TP53* and *SMAD4* mutations significantly increased

with progression of BE to high-grade dysplasia or EAC.

ARID1A is another key molecule driving BE to EAC.[53] *ARID1A* is a member of SWI/SNF family of chromatin remodeling. This molecule has been examined mainly in gastric cancer and reported to be associated with microsatellite instability.[54,55] *ARID1A* mutation was detected around 15% of BE with high-grade dysplasia and EAC. The frequency of loss of ARID1A by immunohistochemistry correlated with disease progression from BE to EAC. The EAC cell line, OE33, showed phenotypes of increased proliferation and aggressive invasion, as the gastric cancer cell line also did.[53,54] In addition to *ARID1A*, the other members of chromatin remodeling factors encoding genes, *ARID2,* and *SMARC4A* mutations, were also reported.[56]

Rho family GTPase activation is an important molecule in gastric cancer and EAC. Rho family consists of Cdc2, Rac1, and RhoA. These molecules are master regulators of actin cytoskeleton rearrangements, promote cancer cell invasion, and cell survival. In gastric cancer, a mutation of *RhoA* is frequently associated with diffuse-type gastric cancer. It has been reported that mutations in *ELMO1* and *DOCK2* are frequently noted in cases with EAC. These are intracellular mediators of RAC1. ELMO1 and DOCK2 promote tumor cell invasion and seem to be associated with EAC carcinogenesis.[57] It was observed that 6% of EAC cases analyzed had mutations in *ELMO1* and 13% in *DOCK2*. Other genes encoding Rac1 activating enzymes were *ECT2* (1%), *TIAM1* (3%), *TRIO* (3%) and *VAV2* (1%) although these frequencies were lower than those in *ELMO*1 and *DOCK2*. Taken together, around 30% of *Rac1-* activating mutations occurred in EAC patients. Also reported in EAC were frequent transversions of A to C at AA sites (T to G at TT sites).[56,58] One possible explanation was that low pH due to GERD induces 8-OH-dG, resulting in A to C transversion at AA sites.[59,60] Further studies also needed to clarify this interesting finding.

Epigenetic Changes and microRNA Status in BE and EAC

Recent global methylation profiling revealed that broad epigenetic alterations occur in both BE and EAC and are associated with carcinogenesis in EAC.[61-64] CpG island promoter hypermethylations are a common feature of cancer, and regulate (traditionally down-regulate) downstream gene expression. On the other hand, DNA hypomethylation increases gene expression.[62] As for specific CpG island promoter methylations, *CDKN2A, APC, CDH1, MGMT, TIMP-3* and *ESR1* have been evaluated in several reports.[51,65-68] *CDKN2A* hypermethylation has been considered to occur in early steps in EAC carcinogenesis. One study suggested that 4 genes, *SLC22A18, PIGR, GJA12* and *RIN2*, were highly methylated in EAC compared to BE.[63]

Table 1: Major molecular alterations reported across malignant progression of BE

Morphological status	Key molecular alterations
BE	SHH, BMP4, SOX9 and CDX3
Esophageal adenocarcinoma	Loss of function of CDKN2A or TP53 (by loss of heterozygosity, or mutation); ARID1A, SMAD4

SHH: Sonic hedgehog; BE: Barrett's esophagus; BMP: Bone morphogenetic protein; SOX9: SRY (sex determining region Y) box 9

Micro RNA (miRNA) is a small non-coding RNA related to post-transcriptional gene expression and silencing. Generally, up-regulation of oncogenic-miRNA or down-regulation of tumor-suppressor miRNA is identified as tumor-related miRNAs. Mir-21 up-regulation has been observed in BE and EAC compared with normal squamous cell epithelium and was associated with carcinogenesis.[69] miRNA-194 was also induced in BE and EAC and found to be related to intestinal metaplasia and metastasis.[70,71] miRNA-143, which suppresses transcription of *KRAS*, was down-regulated in EAC and associated with *TP53*.[72,73] miRNA-31 and miRNA-375 were found to be down-regulated in EAC and are early and late-stage markers of EAC carcinogenesis.[74]

Conclusion

Recent advances of next-generation sequencing have provided researchers with better understanding of genetic and epigenetic alterations in EAC carcinogenesis. However, little study has examined those genetic and epigenetic alterations in combination with clinicopathological factors. In order to elucidate the pathogenesis of BE and EAC and to find molecules for biomarkers and targeting therapy, it will be necessary to analyze those genetic alterations in combination with clinical data in a large-scale cohort.

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Conflicts of interest

There are no conflicts of interest.

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