Mini-Review

Viruses, Other Pathogenic Microorganisms and Esophageal Cancer

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Key Words

Bacterial biota · Epstein-Barr virus · Esophageal cancer · Esophageal squamous cell carcinoma · Human papillomavirus

Abstract

Background: Esophageal cancer (EC) is the eighth most prevalent malignant tumor and the sixth leading cause of cancer mortality throughout the world. Despite the technical developments in diagnosis and treatment, the 5-year survival rate is still low. The etiology of EC remains poorly understood; multiple risk factors may be involved and account for the great variation in EC incidence in different geographic regions. **Summary:** Infection with carcinogenetic pathogens has been proposed as a risk factor for EC. This review explores the recent studies on the association of human papillomavirus (HPV), Epstein-Barr virus (EBV), *Helicobacter pylori* and esophageal bacterial biota with EC. **Key Message:** Among the above-mentioned pathogens, HPV most likely contributes to esophageal squamous cell carcinoma (ESCC) in high-risk populations. New techniques are being applied to studies on the role of infection in EC, which will inevitably bring novel ideas to the field in the near future. **Practical Implications:** Multiple meta-analyses support the finding of a higher HPV detection rate in regions associated with high risk for ESCC compared to low-risk areas. A potential role of HPV in the rise of esophageal adenocarcinoma (EAC) was proposed recently. However, further studies are required before a firm conclusion can be drawn. Less work has been done in studying the association between EBV and ESCC, and the results are quite controversial. *H. pylori* infection is found to be inversely related to EC, which is probably due to the reduced incidence of gastroesophageal reflux disease. Analysis of the esophageal bacterial biota revealed distinct clusters of bacteria in normal and diseased esophagi. A type II microbiome rich in Gram-negative bacteria potentially contributes to EAC by inducing chronic inflammation. Novel findings from such studies as these may benefit public health by justifying anti-infection measures to prevent EC.

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Introduction

Esophageal cancer (EC) is the eighth most prevalent malignant tumor and the sixth leading cause of cancer mortality throughout the world, with about 500,000 new cases diagnosed each year, and it causes an estimated 406,000 deaths annually [1]. Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two main types of EC. ESCC is the predominant subtype, but the incidence of EAC has been rising in developed countries in recent decades [2]. The 5-year survival rate of patients with early-stage tumors is >90% after routine treatment [3]. However, the survival rate dramatically decreases in late-stage patients [4]. Owing to the fact that the majority of patients present to hospital at a late stage, the overall 5-year survival rate for EC is only about 20% [5].

The etiology of EC remains poorly understood; a multifactorial etiology may account for the great variability of EC incidence among different ethnic groups and geographic regions [1, 6]. Epidemiological studies have revealed that tobacco and alcohol consumption are the major risk factors for ESCC [7]. However, additional risk factors include poor oral hygiene, malnutrition and infection. Infections with viruses and bacteria, such as hepatitis B and *Helicobacter pylori*, have been shown to be strong risk factors for certain types of cancer. About 16% of cancer cases on average can be attributed to infections, and the frequency is higher in less developed regions than in more developed regions [8]. The role of infection in the development of EC has long been suspected. For example, the first report of an association between human papillomavirus (HPV) infection and ESCC dates back to the early 1980s [9]. However, a firm link between infection and EC development is still missing, despite a large number of studies on this topic. Herein we will review the recent progress in the field, with a focus on the major tumor-causing agents HPV, Epstein-Barr virus (EBV) and *H. pylori*. The most recent understanding of the role of the esophageal bacterial biota in esophageal disease is also included.

HPV and EC

HPV Biology

HPV belongs to the papillomaviridae, a large family of epitheliotropic DNA viruses with over 150 genotypes. They are non-enveloped DNA viruses containing a double-stranded circular DNA genome of ~8 kb. HPV infection was first shown to be correlated with cervical cancer development in the 1980s, and its role in squamous cell carcinomas of the anogenital tract and a fraction of oral carcinomas is well accepted. Nowadays, HPV infection is considered to contribute to approximately 5% of all human cancers [10, 11]. Based on the ability of HPVs to transform host cells, the minority of HPV types that infect mucosal tissues and the genital tract are subdivided into high-risk and low-risk categories. The high-risk HPV (hrHPV) types, such as HPV16 and HPV18, have the ability to cause human cancer. In contrast, low-risk HPV types, such as HPV6 and HPV11, cause benign genital warts, but not cancer [12–14].

The HPV genome is organized into three distinct regions, namely the early, late and a long control region [14]. The early region encodes viral non-structural proteins including E1, E2, E4, E5, E6 and E7. The late region encodes L1 and L2 open reading frames that can produce major (L1) and minor (L2) capsid proteins for virion assembly [14]. HPV gene expression and the viral life cycle are tightly controlled by epithelial cell differentiation [15]. It is postulated that scratching of the epithelial tissue allows the virus to infect undifferentiated cells in the basal layers of stratified squamous epithelium. Virus early gene expression is initiated in undifferentiated or intermediately differentiated keratinocytes, but late capsid proteins required for virion assembly are only expressed in keratinocytes undergoing terminal differen-

tiation near the apical surface of the epithelium [15]. Early proteins E1 and E2 mainly play roles in regulating viral gene transcription and virus replication, while the E5, E6 and E7 of hrHPVs contribute to virus-induced cellular transformation [14, 16].

HPV Oncogenesis

Professor Harald zur Hausen made the first discovery that HPV was related to cancer biology more than 30 years ago, and jointly won the Nobel Prize for the discovery of the HPV cervical cancer virus in 2008 [17, 18]. It has been well accepted that HPV causes all uterine cervix cancers. Data from the International Agency for Research on Cancer (IARC) demonstrated that HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66 can result in cervical cancer [19]. Among these, HPV16 and HPV18 are regarded as related to 70% of all cervical cancer cases [19].

Integration of viral DNA into the host genome is one of the factors associated with carcinogenesis. Many cervical carcinomas caused by hrHPV contain integrated viral genome fragments. Integration has the potential to upregulate cellular oncogene expression, which may contribute to oncogenesis [20]. Integration of the HPV genome often disrupts expression of the repressive E2 gene and thus leads to abnormal expression of E6 and E7, which are very potent oncoproteins [21]. HPV-positive cervical cancer cell lines show almost strict reliance on these two oncoproteins (sometimes also including E5) to maintain their transformed phenotype.

The hrHPV E7 protein interacts with tumor suppressor pRB and targets it for proteasome-dependent degradation, which eliminates a cell cycle brake and stimulates differentiated epithelial cells to re-enter the cell cycle for continuous proliferation. p53 is another tumor suppressor that senses abnormal cell cycle progression. Its activation by oncogene-induced stress functions as a checkpoint to prevent transformation by inducing cellular senescence or apoptosis. In concert with its associated protein E6AP, a cellular ubiquitin ligase, hrHPV E6 targets p53 for degradation. The hrHPV E6 also upregulates telomerase expression, which renders transformed cells able to replicate perpetually [22, 23].

Detection of HPV in EC

In 1982, Syrjänen [9, 24] first suggested the possibility that HPV might play an etiologic role in the progress of both benign and malignant squamous cell carcinoma of the esophagus; an indirect immunoperoxidase-peroxidase-antiperoxidase technique was used. This opened up a new area of HPV research. Many techniques were used to find evidence of the involvement of HPV in ESCC. Filter in situ hybridization (ISH) was commonly used in the late 1980s but was abandoned because of low sensitivity and poor specificity [25]. PCR and real-time quantitative reverse transcription PCR (real-time qRT-PCR) are the most commonly used techniques. They are fast and convenient methods for identifying HPV DNA and its types, and viral load can be analyzed by real-time qRT-PCR [26–30]. Other methods involved in studies of HPV and ESCC include ISH and enzyme-linked immunosorbent assay (ELISA) [27, 31]. However, techniques based on high-throughput analysis are developing rapidly; gene chips and genome-wide association studies are also used in research [32, 33]. Moreover, meta-analysis, a type of data analysis based on the results of previous studies, has been used to investigate the prevalence of HPV in ESCC [34–36].

HPV and ESCC

There have been a wide range of studies on this topic from various countries, including China, Korea, Iran, India, the United States and Australia. However, in different researches the frequency of HPV infection varies from 0 to 88% in patients with esophageal pre-malignant lesions or carcinomas [36]. Thus, the association of HPV and ESCC is still controversial.

Evidence of a Positive Association between HPV and ESCC

In studies reported from 1992 to 2013, the prevalence of HPV in patients with ESCC was found to be higher in Asia (26.3%) than in Europe and America (14.0%). Studies from high-risk regions in China (Anyang, Xinjiang, Sichuan) indicated that there was strong evidence of an association between HPV and ESCC [31, 32, 37]. He et al. [31] tested samples from 1,435 patients with ESCC from Anyang (China); the serological results demonstrated that the presence of HPV16 E7 was associated with an increased risk of ESCC. Chen et al. [32] screened HPV types associated with ESCC of Kazakh from Xinjiang (China) by gene chip; their results showed that the rate of infection with HPV was 66.67% in the ESCC group, while that of HPV16 was 97.72% and the rate of co-infection with HPV16 and HPV18 was 2.27%. A hospital-based case-control study of 99 patients with ESCC and 100 healthy controls in an Australian cohort suggested that HPV may be an additional risk factor in the multifactorial etiology of EC, although a larger, better-powered study is needed [29].

Evidence of a Negative Association between HPV and ESCC

Some studies have shown no evidence of HPV infection in patients with ESCC. Antunes et al. [28] and Herbster et al. [38] from Brazil as well as Koh et al. [39] from the Korean Cancer Centre Hospital found that tests for HPV DNA were negative in all their samples. Lofdahl et al. [40] set up a population-based study relating HPV infection to tumor site in patients diagnosed with ESCC in Stockholm County in Sweden in 1999–2006. They found that among 204 patients, 10% had tumors harboring HPV DNA, and tumors containing HPV were not overrepresented in the upper compared with the middle or lower third of the esophagus. The authors concluded that HPV was of uncertain oncogenic relevance in ESCC, and they did not demonstrate that HPV was more strongly associated with proximal than distal tumors. An InterSCOPE collaboration study carried out in 2012 examined samples from six countries/regions for multiple HPV serological markers and analyzed their association with ESCC; they concluded that there was 'limited serological evidence' for an association between HPV infection and ESCC [41].

HPV and EAC

The incidence of EAC has increased dramatically in developed countries in the past two decades. HPV infection is believed to be responsible for the dramatic rise in head and neck cancers in western countries, which leads to a suspicion that HPV may also play a role in EAC. However, very few investigations of an association between HPV and EAC have been reported, and no evidence of association was found according to these reports [42]. Recently, Rajendra and colleagues [42, 43] reported for the first time that transcriptionally active hrHPV was strongly associated with EAC. They also showed that increasing viral load and active virus transcription were significantly associated with disease severity in the Barrett's metaplasia-dysplasia-adenocarcinoma pathway. Their findings justify further research on this issue.

Analysis Based on Previous Studies: Meta-Analysis

In order to determine the extent of the association between HPV infection and ESCC on a global scale, many reviews published in 2013 and 2014 have summarized previous studies using meta-analyses. In table 1 we show data from six reviews based on meta-analysis. There are multiple methods of detection with different sensitivities for HPV detection. Most research has been based on PCR, and previous studies have shown that PCR and ISH are almost equivalent in HPV detection [44]. Table 1 suggests a lower prevalence in studies using ISH than in those using PCR. However, use of immunohistochemistry (IHC) and serology led to a higher prevalence of HPV in ESCC than was found by PCR. All six reviews summarized the prevalence of HPV in ESCC, and four of them also calculated the odds ratio (OR) by analysis of case-control studies [35, 36, 45, 46]. For the meta-analyses, the lowest number of studies selected

Table 1. Meta-analysis based on reviews published in 2013 and 2014

Reference	Studies	Cases	Total	Regions	Detection methods		
					HPV type	PCR-based	non-PCR-based
Li et al. [36], 2014	76 ^a	8,990 ESCCs	22.2 (18.3–26.7)	Asia: 26.3 (2.13–4.80)	HPV16	2.9 (1.7–4.8)	24.4 (17.4–33.2)
	case-control: 19 ^b	2,028 ESCCs 2,216 controls	3.32 (2.26–4.87)	Asia: 3.20 (2.13–4.80)	HPV16	4.33 (2.36–7.96)	3.17 (2.12–4.76)
Yong et al. [45], 2013	68 ^a	5,755 ESCCs	13.49	HPV16 Asia: 16.34 (9.97–23.84) HPV16 Africa: 5.14 (0.03–15.53)	HPV16	11.67 (7.74–16.21)	1.82 (0.90–2.95)
	case-control: 10 ^b	1,130 ESCCs 1,614 controls	4.8 (0.27–90.24)	HPV16 Asia: 4.5 (2.39–8.64) HPV16 Africa: 4.97 (0.27–90.24)	HPV16	3.55 (2.05–6.14)	1.25 (0.46–3.43)
Liyanage et al. [46], 2013	21 ^a	1,223 ESCCs	35	high-risk ESCC regions: 2.65 (1.80–3.91)	low- to medium-risk ESCC regions: 4.65 (2.47–8.76)		
	case-control: 21 ^b	1,223 ESCCs 1,415 controls	3.04 (2.20–4.20)	high-risk ESCC regions: 2.65 (1.80–3.91)	low- to medium-risk ESCC regions: 4.65 (2.47–8.76)		
Hardefeldt et al. [35], 2014	132 ^a	27.4 (21.2–28.8)	27.4 (21.2–28.8)	developing countries: 31.1 (25.5–37.4) Asia: 27.9 (22.6–33.8) Africa: 42.4 (34.0–51.3)	developed countries: 18.9 (14.8–23.8) Europe: 18.8 (12.8–26.7) North America: 15.2 (6.5–31.7) South America: 16.3 (7.4–32.2)	16.8 (12.9–21.7)	8.1 (5.7–11.4)
	case-control: 61 ^b	9,291 ESCCs	2.69 (2.05–3.54)	developing countries: 2.92 (2.08–4.11) Asia: 2.94 (2.16–4.0)	developed countries: 2.28 (1.40–3.73) Europe: 1.64 (0.90–2.99) South America: 1.28 (0.07–23.82)	2.35 (1.73–3.19)	1.28 (0.87–1.88)
Petrick et al. [48], 2014	124 ^a	13,832 ESCCs	22.7 (23.4–32.0)	Asia: 31.4 (27.4–35.4) Africa: 19.8 (7.5–32.2)	Europe/Australia: 15.6 (7.3–23.8) North America: 16.6 (3.6–29.6) South America: 19.4 (4.2–34.6)	27.7 (23.4–32.0)	20.5 (1.45–2.90)
	case-control: 61 ^b	13,832 ESCCs	22.7 (23.4–32.0)	Asia: 31.4 (27.4–35.4) Africa: 19.8 (7.5–32.2)	Europe/Australia: 15.6 (7.3–23.8) North America: 16.6 (3.6–29.6) South America: 19.4 (4.2–34.6)	27.7 (23.4–32.0)	20.5 (1.45–2.90)
Syrjänen and Syrjänen [47], 2013	39 ^a	427 ESCCs	39.0 (28.1–51.2)	Other Asia: 28.7 (7.6–66.3)	Europe: 35.3 (22.0–51.4) North America: 61.4 (32.9–83.8) South America: 66.7 (25.6–92.1)	37.2 (23.1–53.9)	histological biopsy: 67.0 (26.1–92.1) IHC: 68.6 (30.8–91.5) ISH: 22.1 (10.0–42.2)
	case-control: 19 ^b	427 ESCCs	39.0 (28.1–51.2)	Other Asia: 28.7 (7.6–66.3)	Europe: 35.3 (22.0–51.4) North America: 61.4 (32.9–83.8) South America: 66.7 (25.6–92.1)	37.2 (23.1–53.9)	histological biopsy: 67.0 (26.1–92.1) IHC: 68.6 (30.8–91.5) ISH: 22.1 (10.0–42.2)

^a Values are given as prevalence (%) with 95% confidence interval in parentheses. ^b Values are given as OR with 95% confidence interval in parentheses.

was 21 [46] and the number of ESCC cases was at least 427 [47]; the highest number of studies selected was 132 [35] and the number of ESCC cases included was 13,832 [48]. In table 1, the prevalence of HPV in ESCC given by each meta-analysis is seen to range from 13.49 to 39%, which is lower than that of cervical squamous cell carcinoma (86.9%) [36]. The summary OR varied from 2.69 to 4.80 (mean 3.46); these ORs suggest an approximate threefold increased risk of ESCC in the presence of HPV.

Table 1 also depicts the subgroup analyses of HPV in patients with ESCC, indicating variation with geographic area, HPV type and detection method. The prevalence in developing countries is 12.2% higher than in developed countries, and the OR indicates a greater risk in developing countries, which is in accordance with prevalence data [35]. Africa has the highest prevalence, at 42.4%, among all regions in the study of Hardefeldt et al. [35]; however, the prevalence was 19.8% and a surprising 5.14% in other two reviews [45, 48]. In a meta-analysis from Asia, in which the largest population was Chinese (in more than half of the studies included), the prevalence ranged from 16.34 to 31.4% [35, 36, 45, 48]. From the point estimate of risk, Asia (ORs 3.2–4.5) has a significantly higher risk of HPV in patients with ESCC than Europe or America (ORs 1.27–2.64) [35, 36, 45].

Given that HPV16 and HPV18 are the most frequently detected types in HPV-associated cancers, table 1 only displays HPV16/18 subtypes. Yong et al. [45] evaluated the prevalence of HPV16 and HPV18 in ESCC and found 11.67% (HPV16) and 1.82% (HPV18). They also calculated separate ORs for HPV16 (OR 3.55) and HPV18 (OR 1.25) and found that HPV16 was the most frequently observed subtype in ESCC. The prevalence values calculated in two other reviews are in accordance with the report by Yong et al. [35, 36]. Overall, meta-analyses in the reviews involving subgroups with different HPV types indicated that HPV16 is significantly associated with the risk of ESCC.

Association between EBV Infection and ESCC

EBV is a member of the gamma-herpesvirus family which was discovered more than 40 years ago. EBV is a widespread pathogen that is found in all human populations and establishes lifelong asymptomatic latent infection [49]. EBV preferentially infects B lymphocytes and infection of other cell types (e.g. epithelial cells) is less efficient. EBV is able to transform resting B cells into lymphoblastoid cell lines, whereas infection of epithelial cells in vitro does not involve this process. During latent infection, a limited viral genome is expressed, incorporating six EBV nuclear proteins (EBNAs 1, 2, 3A, 3B, 3C and LP) and three latent membrane proteins (LMPs 1, 2A and 2B) [49].

EBV is an oncogenic virus that has been reported to be associated with Burkitt's lymphoma, Hodgkin's disease, breast cancer, nasopharyngeal carcinoma, gastric carcinoma and leiomyosarcoma [50]. However, whether EBV is related to ESCC remains controversial: there are limited studies and their conclusions disagree. Different assays with varying sensitivity and specificity have been used to investigate specimens from different regions or countries. Mizobuchi et al. [51] from Japan examined 41 surgical specimens as well as 12 cell lines of ESCC by amplifying the EBV EBNA-1 gene using PCR and found no EBV genome in cases of EC. Yanai et al. [52], also from Japan, found no EBER-1-positive cancer cells in any EC lesion tested by real-time quantitative PCR. Sunpaweravong et al. [53], from Thailand, found no EBER-positive cancer cells by ISH, but reported five samples in which EBER-positive tumor-infiltrating lymphocytes were found. Wang et al. [54], from northern China, evaluated 51 differentiated tumors by ISH using an antisense probe against EBV-encoded small RNA-1 and PCR amplification for the EBV *Bam*HI W fragment; they found that EBV was negative by both ISH and PCR. In contrast, another study from Taiwan detected EBV DNA by PCR in 11 samples (35.5%) from 31 patients with ESCC.

Expression of EBERs in ESCC was further confirmed with ISH, whereas no LMP-1 expression was detected, indicating that EBV may be associated with ESCC [55]. Jenkins et al. [56] from the USA found EBV in 8.3% (5/60) of cases by PCR. In Germany, Awerkiew et al. [57] confirmed the association. EBV DNA was detected in 35% of squamous cell carcinomas and 36% of adenocarcinomas by nested PCR, which is more sensitive than conventional PCR. The most convincing report is from Shantou, located in South China. EBV EBER and LMP-1 proteins were identified in 10 (6.1%) EC specimens by both ISH and IHC. The 10 EBV-associated carcinomas were all poorly differentiated squamous cell carcinomas or undifferentiated carcinomas accompanied by heavy degrees of lymphocyte infiltration in the tumor stroma. This suggests an association between EBV infection, EC cell differentiation and lymphocyte infiltration [58].

Owing to different detection techniques and geographic factors, the results of such studies are variable even in one country (China). ISH (using an EBER probe) and IHC (using antibodies against LMP-1, etc.) are better than PCR (amplifying the EBNA-1 gene) for investigating the presence of EBV in ESCC because of cross-contamination with tumor-infiltrating lymphocytes surrounding the carcinoma. Considering the contradictory results obtained using ISH, racial and geographic factors seem to play a role in the number of cases detected by different laboratories. Also, ISH may produce false-negative results due to a higher rate of RNA degradation. It would be favorable for the prevention and diagnosis of poorly differentiated or undifferentiated ESCC if the association between EBV infection and lymphocyte infiltration were to be validated.

In summary, despite some negative reports, EBV infection has a relationship with at least some subtypes of ESCC, to some extent, in certain races and countries. More and larger studies are required from other parts of the world, using standardized detection methods, to further elucidate the association between EBV and ESCC.

***Helicobacter pylori* and Esophageal Carcinoma**

H. pylori is the most frequently observed bacterium in reports of gastric carcinoma. As *H. pylori* can induce superficial gastritis, a series of animal and clinical studies have demonstrated that it contributes to the development of gastric carcinoma [59]. More than half of the world's human population has been infected by *H. pylori*, and the proportion infected in developing countries is larger than that in developed countries [60]. In recent years, however, carriage of *H. pylori* has been controlled by the application of antibiotics [61]. This has led to a significant decline in gastric cancer and a dramatic increase in EC [62]. Some investigators believe that the reduction of EC associated with *H. pylori* infection is related to the inhibition of acid production caused by *H. pylori* by its stimulation of atrophic gastritis [63]. The cag+ strains of *H. pylori* are associated with gastric cancer, and they also induce the production of interleukin 8 (IL-8) [64]. This leads to higher levels of IL-1 β and tumor necrosis factor- α (TNF- α) [65]; these two cytokines reduce acid production and therefore decrease the risk of gastroesophageal reflux. Indeed, *H. pylori* infection with atrophy of the gastric corpus can lead to a lower incidence of gastroesophageal reflux disease than reported in patients with increased or even normal levels of acid [66].

Esophageal Biota and Esophageal Disease

Mutualism between the human host and bacterial biota is essential for human health. In luminal organs of the digestive system, a balanced bacterial population may develop the mucosal immune system, maintain the inner environment of these luminal organs or provide

Table 2. Bacterial biota in the esophagus

Patient group	<i>Firmicutes</i>	<i>Proteobacteria</i>	<i>Fusobacteria</i>	<i>Bacteroides</i>	<i>Actinobacteria</i>	TM7
Normal	40%	49%	–	8%	3%	–
Reflux esophagitis	33%	43%	10%	10%	2%	2%
Barrett's esophagus	55%	20%	9%	14%	2%	–

specific nutrients [67]. Imbalances in the bacterial biota induce a series of health problems, such as *H. pylori*-induced gastric carcinoma [59]. Therefore, the balance of bacterial biota in the esophagus is important in maintaining the normal physiological environment. Alterations in the bacterial biota may cause esophageal disease and even esophageal tumors.

Traditional culture methods were inappropriate for study of the bacterial biota of the esophagus. The appearance of metagenomics techniques in recent years has increased understanding of the microbial systems in the esophagus [68]. Unlike traditional culture methods, metagenomics does not rely on microbial clonal cultures; DNA samples are obtained directly from the environment being studied. Specific genes, such as the 16S rRNA gene, are sequenced to construct a library or compared with known libraries [69]. After a series of organized analyses, a great deal of information about the genetic material can be obtained and the database can be used as a reference for future work.

Metagenomics has been used to investigate the composition of the bacterial flora of the esophagus. In a study performed in 2004, broad-range 16S rRNA PCR was used to analyze esophageal biopsies from four human adults with normal esophageal mucosa [70]. The data showed that the bacterial biota contained six phyla. *Firmicutes*, accounting for 626 of 900 clones (69.6%) was the most common phylum, the others were *Bacteroides* (20.2%), *Actinobacteria* (4.3%), *Proteobacteria* (2.2%), *Fusobacteria* (2.2%) and TM7 (1.4%). *Streptococcus*, which is a member of the *Firmicutes*, accounting for 39%, was the most common genus. In 2013, a similar study was carried out in 18 patients with a normal or diseased esophagus [71]. The results (table 2) showed differences in the bacterial biota among individuals with a normal esophagus, reflux esophagitis and Barrett's esophagus. Both reflux esophagitis and Barrett's esophagus are closely associated with EC.

In 2009, an analysis of the microbial 16S rRNA genes in esophageal biopsies from 34 patients with normal or diseased esophagus classified the microbiome of the esophagus into two types [72]. The type I microbiome mainly comprises *Streptococcus* and is related to the normal esophagus. The type II microbiome includes many Gram-negative microaerophiles or anaerobes and has a closer relationship with esophageal disease. The type II microbiome may affect the health of the esophagus through two mechanisms that differ from type I. One of those mechanisms is associated with a higher incidence of gastroesophageal reflux disease. The other mechanism is related to the lipopolysaccharide in the cell walls of Gram-negative bacteria. Lipopolysaccharide can induce the nitric oxide synthase pathway, which relaxes the lower esophageal sphincter [73]. This contributes to gastroesophageal reflux, which is associated with a higher incidence of EAC.

Bacterial Infection and Signal Transduction

As previously stated, the bacterial population may aid development of the mucosal immune system, but it may also induce disease. These processes involve the participation of many signaling pathways.

Cellular receptors and signal factors play important roles in the interaction of bacterial infections and signal transduction in epithelial cells. In luminal organs of the digestive system,

bacterial populations may change the levels of expression of signal factors or cellular receptors to influence the health of these organs [74]. For example, some bacterial strains, such as *H. pylori*, increase the expression of IL-1 β , and cells expressing the IL-1 β receptor gene may be induced to express inflammatory cytokines [75].

Many epithelial cells produce a type of glycoprotein, called mucin, to protect their surfaces [76]. Some mucins may act as cellular receptors to bind signal factors, such as IL-1, IL-4, IL-6 and TNF- α , and therefore influence the inflammatory response. Most are secreted as signal factors to control IgA levels in order to regulate the inflammatory response. Mucins may be affected by bacteria; in particular, MUC2 and MUC5AC can be unregulated by both Gram-negative and Gram-positive bacteria [77]. In addition to their involvement in inflammation, mucins may also contribute directly to tumorigenesis [43]. In one study, MUC2 knockout reduced the size of bowel carcinomas in mice [78]. Mucins can interact with antiapoptotic proteins, such as the trefoil family of peptides, and with the epidermal growth factor receptor family, for example the human epidermal growth factor receptor 2 [79]. This leads to their influence on cell growth and tumorigenesis [76].

In the normal esophagus, mucins are not expressed in squamous mucosal cells. However, in a study in 2006, antibodies against each member of the mucin family were used to stain mucosal biopsies from 35 patients with Barrett's esophagus. It was found that all samples were stained by MUC2, MUC3 and MUC5AC antibodies, and 40 and 90% of samples from patients with non-dysplastic Barrett's esophagus were stained by MUC1 and MUC6 [80]. Therefore, mucins may play important roles in tumorigenesis in the esophagus.

Conclusions

EC is one of the most serious malignancies of the gastrointestinal tract. The incidence rate of EC varies dramatically among different ethnic groups and geographic regions. Many risk factors, including smoking, alcohol consumption, poverty and infection, may be involved in EC development. Although a clear-cut association between infection and EC is still missing, there is evidence that HPV infection may contribute to ESCC in certain high-risk populations. Well-controlled studies in large cohorts with more advanced techniques are required. In addition, new techniques are emerging that allow systematic study of the esophageal microbiota and their role in disease. A distinct cluster, the type II microbiome that may contribute to EAC, has been reported. Novel findings from more advanced studies may benefit public health by justifying anti-infection measures, such as HPV vaccination, to prevent EC.

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