79864_Auto_Edited.docx

WORD COUNT	3084	TIME SUBMITTED	26-OCT-2022 02:48PM
		PAPER ID	91948636

Name of Journal: *World Journal of Gastroenterology*
Manuscript NO: 79864
Manuscript Type: MINIREVIEWS

Development of EBV-associated gastric cancer: infection, inflammation, and oncogenesis

Development of EBV-associated gastric cancer

Hisashi Iizasa, Andi Visi Kartika, Syntayehu Fekadu, Shunpei Okada, Daichi Onomura, Afifah Fatimah Azzahre Ahmad Wadi, Mst. Mahmuda Khatun, Thin Myat Moe, Jun Nishikawa, Hironori Yoshiyama

Abstract

EBV-associated gastric cancer (EBVaGC) cells originate from a single-cell clone infected with EBV. However, more than 95% of patients with gastric cancer have a history of *Helicobacter pylori* (*H. pylori*) infection, and *H. pylori* is the major causative agent of gastric cancer. Therefore, it has long been argued that *H. pylori* infection may affect the development of EBVaGC, a subtype of gastric cancer. Atrophic gastrointestinal inflammation, a symptom of *H. pylori* infection, is found in the gastric mucosa of EBVaGC. Therefore, it remains unclear whether *H. pylori* infection is a cofactor for gastric carcinogenesis caused by EBV infection or whether *H. pylori* and EBV infections act independently on gastric cancer formation. It has been reported that EBV infection assists the oncogenesis of gastric cancer caused by *H. pylori*infection. In contrast, several researchers have reported that *H. pylori* infection accelerates tumorigenesis initiated by EBV infection. By reviewing both clinical epidemiological and experimental data, we have reorganized the role of *H. pylori* and EBV infections in gastric cancer formation.

Key Words: Helicobacter pylori; Epstein-Barr virus; Epstein-Barr virus-associated gastric cancer; coreceptor; inflammation; oncogenesis

Iizasa H, Kartika AV, Fekadu S, Okada S, Onomura D, Wadi AFAA, Khatun MM, Moe TM, Nishikawa J, Yoshiyama H. Development of EBV-associated gastric cancer: infection, inflammation, and oncogenesis. *World J Gastroenterol* 2022; In press

Core Tip: Epstein-Barr virus-associated gastric cancer (EBVaGC) tumor cells originate from a single cell clone infected with EBV. In contrast, it is reported that more than 95% of patients with gastric cancer have a history of H. pylori infection. Accordingly, it has long been argued that H. pylori infection may have some effect on the development of EBVaGC, a subtype of gastric cancer. It is also a mystery that the number of gastric cancer patients is higher in Asia, South America, and the Middle East. We will reorganize the role of H. pylori and EBV infections in gastric cancer formation.

INTRODUCTION

Epstein-Barr virus (EBV)-associated gastric cancer (EBVaGC) accounts for 10% of all gastric cancers. At the same time, more than 95% of patients with gastric cancer have a history of *Helicobacter pylori* (*H. pylori*) infection. Thus, the question arises whether *H. pylori* and EBV infections promote gastric cancer formation in a dependent or independent manner. The high prevalence of gastric cancer in Asia, South America, and the Middle East is also an intriguing fact.

EPSTEIN-BARR VIRUS IS AN ONCOGENIC HUMAN HERPESVIRUS

EBV infects B lymphocytes and epithelial cells and is an oncogenic virus that assists the proliferation of latently infected cells, resulting in the development of Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, and EBV-associated gastric cancer (EBVaGC) [1].

More than 90% of adults are latently infected with EBV; however, cytotoxic T lymphocytes that recognize EBV antigens suppress the proliferation of viral antigen-positive cells. When the local or systemic immune function is compromised, EBV-positive cells begin to proliferate. B lymphocytes that migrate to local areas where immune surveillance is weak often transit to lytic infection, resulting in viral production. Under such conditions, EBV appears to be transmitted to and infects gastric epithelial cells. The expression of EBV genes causes epithelial cells to acquire proliferative properties and resist apoptosis, and cells that escape immunological elimination may start proliferating [2].

EBV-ASSOCIATED GASTRIC CANCER

Molecular features

Classification of gastric cancer by molecular mechanism was performed by exhaustive analysis of next-generation sequencing data from a large number of cases, resulting in division of gastric cancer into four molecular subtypes, namely microsatellite instability (MSI), chromosomal instability (CIN), genomically stable (GS), and EBV [3]. These classifications have facilitated the identification of specific

therapeutic candidates for each subtype of gastric cancer, and revealed that each of these four subtypes is driven by a specific developmental mechanism that needs to be clarified individually. In particular, the molecular biology of EBVaGC is characterized by frequent and extensive methylation of the promoter regions of tumor cell genes [4]. *De novo* EBV infection induces DNA methylation in more than 3000 gene promoter regions within 4 wk [4]. However, methylation of the promoter of the mismatch repair gene MLH1, which is frequently observed in microsatellite instability (MSI), is not observed in EBVaGC [5]. In addition to inactivation by DNA methylation, EBV genome binds to heterochromatin, a region of inactivation, causing aberrant activation of the region (enhancer infestation) and increases the expression of surrounding proto-oncogenes [6].

Clinical features

In EBVaGC, which accounts for 5–10% of all gastric cancers, all tumor cells are infected with EBV. Endoscopy is the most informative means of diagnosing gastric cancer. EBVaGC is observed as a superficial depressed lesion in the upper part of the stomach. Using endoscopic biopsy specimens, EBV-encoded RNA in situ hybridization (EBER-ISH), stains all gastric cancer cells positive for EBER, even at the stage of intramucosal cancer [7]. The histological hallmark of EBVaGC is lymphoepithelioma-like carcinoma, in which a diffuse lymphocytic infiltrate is observed around EBER-positive epithelial tumor cells [8]. Furthermore, EBVaGC tumor cells are derived from the proliferation of a single EBV-infected epithelial cell [8, 9].

Many studies have shown a male predominance (2-fold) of EBVaGC, suggesting that the risk may exist in male lifestyle and occupational factors [10]. The percentage of EBVaGC to total gastric cancer is higher in younger patients. In men, the proportion of EBVaGC decreases with increasing age, especially in pyloric gastric cancer. In women, the decrease in the proportion of EBVaGC with increasing age is not clear. Consumption of salty foods that cause mechanical damage to the gastric epithelium, exposure to wood and iron filings are associated with a higher EBVaGC risk [11].

EBVaGC is a gastric cancer with a relatively good prognosis. A Dutch study explained that EBVaGC is characterized by fewer lymph node metastases, less residual disease, and younger patient age, which results in longer disease-free survival [12]. Cohort study data from TCGA also reported that compared to MSI, GS, and CIN subtypes, EBVaGC has the best recurrence-free period and overall survival [13].

EBVaGC tumors are frequently found in the non-antral part of the stomach [10, 14]. On the other hand, *Helicobacter pylori* (*H. pylori*)-associated gastric cancer mostly occurs in the antral region [10]. Because moderate to severe atrophic gastric mucosa due to *H. pylori* infection was characteristically observed surrounding early gastric cancers, gastritis may play an important role in the tumorigenesis of EBVaGC [14]. Development of gastric cancer is supposed to follow the "infection, inflammation, and carcinogenesis" route, which consists of infection with *H. pylori*, chronic gastritis, intestinal metaplasia, and cancer. In contrast, in the case of EBVaGC, it is controversial whether tumor formation is initiated by EBV-infected normal mucosal cells or promoted by EBV-infected cells in precancerous lesions [15]. Abe *et al* performed EBER-ISH on 1,110 sections of non-neoplastic gastric mucosal tissue from 300 cases and found 2 (0.18%) ductal-level EBER-positive lesions [16].

Mutual contribution between EBV and *H. pylori* in the carcinogenesis will be discussed later in Chapter 6.

EBV infection of epithelial cells

EBV infects B lymphocytes through the binding of the viral glycoprotein, gp350, to the high-affinity receptor CD21, followed by the binding of gp42 to HLA class II molecules, resulting in membrane fusion [17]. In contrast, when low-affinity co-receptors are used to infect CD21-negative epithelial cells, the infection efficiency is extremely low (**Fig. 1**).

The CD21-independent routes of epithelial cell infection include the following: (1) The viral envelope glycoprotein gp350/220 binds to CD35. (2) Integrins αVβ5, αVβ6, and αVβ8 interact with the viral envelope glycoprotein gH/gL complex to fuse the viral

envelope with the epithelial cell membrane. (3) The BMRF2 membrane protein, which is expressed during EBV lytic infection, binds to α3, α5, αV, and β1 integrins. (4) EphA2 and NMHC-IIA bind to gH/gL produced by many herpesviruses and enhance infection efficiency.

A previous study reported that a boy with X-linked agammaglobulinemia who did not have mature B lymphocytes due to a genetic enzymatic deficiency did not develop EBV infection [18]. EBV infection of epithelial cells was considered to occur after EBV infection of B lymphocytes, because the epithelial cells of the affected boy were intact. It is believed that the EBV-infected B lymphocytes carry and deliver EBV to epithelial cells via cell-to-cell transfer. In the case of CD21-independent infection, the efficiency of epithelial cell infection by cell-to-cell transfer is more than 1,000 times higher than that of direct epithelial cell infection by EBV particles [19]. It is speculated that the infection of epithelial cells *via* B lymphocytes is promoted when viral activation and lymphocyte infiltration are accompanied by inflammation (**Fig. 1**).

EBV-associated gastric cancer-derived cell lines

In EBVaGC, all tumor cells are infected with EBV. However, cell lines established from gastric cancer tissues, similar to those in nasopharyngeal carcinoma, are almost EBV-negative [20]. The EBV genome in EBVaGC tumor cells exists as a plasmid-like episome that does not integrate into the host chromosomes. However, the presence of the virus did not appear to favor cell growth *in vitro*. Rather, it may be more convenient for *in vitro* cell growth not to use extra energy to maintain the episomes. Alternatively, expression of viral genes, such as microRNAs, may be crucial for tumor cells to evade elimination by the *in vivo* immune system. In fact, EBV-positive KT cells established from EBVaGC can only be passaged by transplantation into SCID mice and cannot be expanded in an *in vitro* culture system [21]. SNU-719, YCCEL1, and NCC-24 are rare cells established from EBVaGC that can be propagated *in vitro*. These cell lines appear to be unique, because the presence of EBV episomes is essential for their growth.

Experiments with hydroxyurea and EBNA1 siRNAs were not successful in shedding the EBV episome from SNU-719 cells [22].

Gastric epithelial cell lines infected with recombinant EBV

We established gastric epithelial cells which are infected with recombinant EBV, where a drug-resistant gene was inserted into the nonessential BXLF1 (thymidine kinase) gene (**Fig. 2**). It was possible to elucidate the oncogenic molecular mechanism of EBV-infected epithelial cells by comparing EBV-positive cells with EBV-negative cells. EBV infection was found to markedly promote the proliferation of gastric epithelial cells [23].

The EBV-infected gastric epithelial cells also exhibit a type I latent infection that expresses EBNA1 and LMP2A, similar to that in EBVaGC *in vivo*. EBNA1 promotes tumorigenesis by p53 ubiquitination, suppressing TGF-β signaling, and enhancing the transcription of anti-apoptotic protein survivin [24]. In contrast, LMP2A activates PI3K/Akt signaling similar to that by B-cell receptor (BCR) stimulation, increases survivin expression, and resists apoptosis [25]. LMP2A also induces DNA methyltransferases, resulting in epigenetic changes in infected cells [26]. BARF1 is strongly expressed as a latent gene in EBV-associated epithelial tumors [27]. Nasopharyngeal carcinoma-derived cells infected with recombinant EBV constitutively expressing BARF1 exhibit resistance to apoptosis [28].

In addition to the oncogenic activity of EBV proteins expressed in type I latent infection, non-coding RNAs (miRNAs and EBERs) that are not translated into proteins have been investigated. Multiple BART miRNAs cooperatively repress lytic replication [29]. BART miRNAs also downregulate pro- and anti-apoptotic mediators such as caspase 3 [30]. EBERs bind to protein kinase R and disrupt innate immune function [31]. The elimination of EBER2 from the EBV genome reduces the efficiency of B lymphocyte transformation [32].

Inflammation and carcinogenesis

Clinical observation

It is very difficult to collect EBVaGC cases without *H. pylori* infection, because most of gastric cancer patients are infected with *H. pylori* [33, 34]. However, a clinical study was conducted to investigate the relationship between EBV infection, *H. pylori* infection and atrophic gastritis in 468 patients with chronic gastritis [35]. The study confirmed that EBV-positive patients had a lower pepsinogen I / pepsinogen II ratio than EBV-negative patients. EBV infection significantly increased the risk of atrophic gastritis, especially in *H. pylori* negative patients. On the other hand, a report from Mexico mentioned that an EBER1 *in situ* hybridization showed EBV infection of epithelial cells could be detected in gastric cancers as well as in a third of non-atrophic gastritis samples [36]. This paper was able to show that EBV infects early cancer precursor lesions. However, it is difficult to say that EBV causes cancer directly or indirectly by triggering inflammation.

Inflammation and initiation of innate immune mechanisms promote EBV activation, although it is difficult to assess the extent to which these mechanisms are involved in tumorigenesis of EBV-infected cells (**Fig. 3**). EBV proliferation occurs at the early stage of EBVaGC formation, because EA-IgG and VCA-IgG antibodies against early viral antigens and capsids are elevated in the sera of patients with EBVaGC. In addition, while the incidence of EBVaGC is approximately 10% worldwide, the incidence of gastric cancer after surgical invasion by gastric anastomosis increases by three times (30%) [8].

We investigated the relationship between *H. pylori*-associated gastritis and EBV propagation in the stomach. Gastric biopsy specimens were collected from patients with chronic atrophic gastritis and were categorized into three histopathological stages, namely mild, moderate, and severe. The specimens were subjected to DNA extraction and quantitative PCR for quantification of EBV genome copy numbers [37]. More than 900 copies of the EBV genome were frequently detected in patients with moderate atrophic gastritis. In other words, EBV frequently activated proliferation in *H.*

pylori infected patients with moderate chronic atrophic gastritis with strong histological inflammation.

On the other hand, EBVaGC was significantly associated with marked mucosal atrophy and moderate to marked lymphocytic infiltration, but there was no direct association with intestinal metaplasia [7]. Although this would appear to indicate that EBVaGC is not directly associated with H. pylori infection, this result is consistent with our findings. This is because the intestinal metaplastic epithelium resulting from prolonged gastritis must be an unsuitable mucosal environment for the growth of both *H. pylori* and EBV [38].

Experimental observation

Several studies have been conducted on the interaction between EBV and *H. pylori* using gastric epithelial cell lines. Because it is difficult to infect epithelial cells of the two microorganisms simultaneously, experiments have been conducted on sequential infection with EBV first and *H. pylori* second, or vice versa.

Persistent infection of the gastric mucosa with *H. pylori* cagA-positive strains causes gastric cancer. This is because the tyrosine-phosphorylated CagA protein binds to the tyrosine phosphatase SHP2 in gastric epithelial cells, activating *Ras* oncogene. On the other hand, SHP1 that competes with SHP2 weakens the oncogenic activity of SHP2. Saju P. *et al* showed that EBV infection of gastric epithelial cells activates host cell promoter methylation and decreases SHP1 expression [39]. In other words, SHP2 activity becomes relatively higher and EBV infection promotes carcinogenesis of *H. pylori* associated gastric carcinoma. The induction of DNA methylase by EBV infection of gastric epithelial cells also decreases expression of tumor suppressor genes such as APC, breast cancer susceptibility gene 1 (BRCA1), and phosphatase and tensin homolog deleted from chromosome 10 (PTEN) [40].

Furthermore, the activation of innate immune signals by *H. pylori* attachment enhanced the expression of the EBV co-receptor EPHA2 in gastric epithelial cells, thereby increasing the frequency of EBV infection in epithelial cells [41]. Another

study demonstrated that organoids derived from gastric cancer cells but not organoids derived from the normal gastric epithelium were infected by EBV [42]. The probable reason for this is that the gastric organoids maintain cell polarity to express EPHA2 only between cells. Therefore, the localization of EPHA2 might change due to gastric epithelial cell injury caused by *H. pylori* infection or by prior gene mutation, which subsequently facilitates EBV infection.

Tumorigenic mechanism of EBV infected epithelial cells

At present, it is difficult to infect primary gastric epithelial cells with EBV and immortalize them. Instead, gastric epithelial cell lines persistently infected with EBV have been used to elucidate the tumorigenic mechanisms of EBV genes during latent infection.

EBV genes that encode untranslational RNA

The EBV genome contains two miRNA clusters, consisting of four BHRF1 miRNAs and 40 BART miRNAs. Although BHRF1 miRNA is poorly expressed in epithelial cells, BART miRNAs are highly expressed in latently infected epithelial cells and play a substantial role in tumorigenesis [43].

B) Epigenetic changes of gene expression in EBV-infected epithelial cells

Modification of gene expression *via* methylation is frequently observed in EBVaGC. Tumor suppressor genes, such as *p14*, *p16*, *p73*, *PTEN*, *APC*, *RASSF1A*, and *CXXC4*, are induced by promoter methylation, and expression of molecules important for cell invasion, including THBS1, E-cadherin (CDH1), and TIMP2, is repressed by promoter methylation. The decreased expression of these molecules may be involved in carcinogenic processes [44].

Multiple EBV episomal DNAs have been shown to approach enhancer sites in the genome, alter the surrounding chromatin structure (enhancer infestation), and activate

genes such as transcription factors [45]. Although epigenetic analyses have been conducted to understand tumorigenesis, the overall concept remains unclear.

C) Model of tumorigenesis for EBV infection of epithelia

Viral gene products transcribed in cells latently infected with EBV confer resistance to apoptosis. EBV gene products also accumulate mutations in genes of the infected cells. Genetic changes in the infected cells further affect EBV gene expression and alter intercellular communication, including the cross-talk between EBV-infected epithelial cells and immune cells [46] or the epithelial-mesenchymal transition [47]. In other words, changes induced by persistent EBV infection to host cell signaling and host immune responses advance the tumorigenic stage [48].

Future prospects

With the progress in research on EBER, miRNA, and long non-coding RNA (lncRNA), the functions of these molecules in latent EBV-infected cells are being elucidated. A highly tumorigenic B81 EBV strain was isolated from a patient with nasopharyngeal carcinoma [49], but an EBV strain unique to gastric cancer has not yet been isolated.

Host gene mutations frequently observed in EBVaGC, including changes in *PIK3CA*, *ARID1A*, *PD-L1*, and *PD-L2*, [3] are considered to affect the histological characteristics, clinical course, and response to treatment. EBV-induced tumorigenesis is believed to be affected by environmental factors, such as previous infections; however, the molecular basis that characterizes EBVaGC remains to be elucidated.

Considering that EBVaGC most strongly expresses PD-L1 and PD-L2 among the four molecular subtypes of gastric cancer, immune checkpoint inhibitors are expected to be effective therapeutic agents for EBVaGC [50, 51]. In addition, *PIK3CA* mutations and *JAK2* amplifications are frequently observed in EBVaGC. Therefore, PI3K inhibitors and JAK2 inhibitors may be effective. Other EBNA-1 inhibitors are also expected to be EBV-specific therapeutic agents [52].

CONCLUSION

Several clinical and experimental data support the etiological role of H. pylori in EBV-associated gastric cancer.

79864_Auto_Edited.docx

ORIGINALITY REPORT

3%
SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	39 words — 1%
2	Kai Zhao, Yu Zhang, Suhong Xia, Lina Feng, Wangdong Zhou, Mingyu Zhang, Ruonan Dong, Dean Tian, Mei Liu, Jiazhi Liao. "Epstein-Barr Virus is Associated with Gastric Cancer Precursor: Atrophic Gastritis", International Journal of Medical Sciences, 2022 Crossref	26 words — 1%
3	C. D. Shannon-Lowe, B. Neuhierl, G. Baldwin, A. B. Rickinson, H.-J. Delecluse. "Resting B cells as a transfer vehicle for Epstein-Barr virus infection of epithelial cells", Proceedings of the National Academy of Sciences, 2006 Crossref	14 words — < 1%
4	pubmed.ncbi.nlm.nih.gov Internet	13 words — < 1%
5	Hisashi Iizasa, Asuka Nanbo, Jun Nishikawa, Masahisa Jinushi, Hironori Yoshiyama. "Epstein-Barr Virus (EBV)-associated Gastric Carcinoma", Viruses, 2012 Crossref	12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS