Epstein-Barr Virus-Associated Gastric Carcinoma: Use of Host Cell Machineries and Somatic Gene Mutations

Hiroyuki Abe, Atsushi Kaneda, Masashi Fukayama

Department of Pathology, Graduate School of Medicine, The University of Tokyo, Tokyo, Department of Molecular Oncology, Graduate School of Medicine, Chiba University, Chiba, and CREST, Japan Science and Technology Agency, Saitama, Japan

Key Words
Epstein-Barr virus · Gastric cancer · DNA methylation · EBNA1 · Lymphoepithelioma-like carcinoma · microRNA · Exosome · Molecular subtype · ARID1A · PIK3CA

Abstract
Epstein-Barr virus (EBV)-associated gastric carcinoma (EBVaGC) is a distinct subtype of gastric carcinoma, consisting of clonal growth of EBV-infected epithelial cells. Its unique characteristics have been demonstrated by epidemiological, clinical and pathological studies using in situ hybridization for EBV-encoded small RNAs. An oncogenic process for EBVaGC has also been revealed. EBV uses various host-cell machineries, including cell division machinery to propagate clonal virus genomes, DNA-methylation machinery to epigenetically control infected cells, and microRNA and exosome machineries to modify the behavior and microenvironment of infected cells. Recent comprehensive molecular analyses from The Cancer Genome Atlas project demonstrate that EBVaGC is a representative molecular subtype that is distinct from microsatellite unstable, genomically stable and chromosome unstable subtypes. In addition to having the highest level of DNA methylation in CpG islands of promoter regions, EBVaGC harbors particular gene alterations, including a high frequency of mutations in PIK3CA and ARID1A, mutation in BCOR, and amplification of PD-L1 and PD-L2. Although currently undetermined, the virus might use the altered cellular functions that are induced by these somatic mutations. Further investigation of virus-driven oncogenesis will enable hitherto unknown functions of stomach epithelial cell machineries to be elucidated, which may reveal potential therapeutic targets for EBVaGC.

Introduction
Gastric cancer is classified into several molecular subtypes. Epstein-Barr virus (EBV)-associated gastric carcinoma (EBVaGC) is a gastric carcinoma subtype resulting from clonal growth of EBV-infected stomach epithelial cells. This subtype has been considered as unique in its histopathological [1] and epigenetic features [2, 3], and, more recently, comprehensive molecular studies demonstrated EBVaGC to have characteristic molecular abnormalities [4, 5]. This review first summarizes the epidemiological, clinical and pathological characteristics of EBVaGC. We then discuss the molecular basis of EBV-induced carcinogenesis, focusing on how the virus uses...
Table 1. Characteristics of EBV-associated gastric carcinoma

<table>
<thead>
<tr>
<th>Epidemiology</th>
<th>Pathology</th>
<th>Molecular biology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Worldwide distribution with no endemic area</td>
<td>High frequency in the proximal stomach</td>
<td>Monoclonal viral genome in each tumor cell</td>
</tr>
<tr>
<td>Male predominance</td>
<td>Lower rate of lymph node metastasis</td>
<td>DNA hypermethylation of viral and host genome</td>
</tr>
<tr>
<td><em>H. pylori</em> infection and smoking as a risk factor</td>
<td>Histology of lymphoepithelioma-like carcinoma or lace pattern</td>
<td>– Viral gene expression: latency I or II (EBNA1, EBERs, LMP-2A, BARTs and BART miRNAs)</td>
</tr>
<tr>
<td>Atrophic gastritis in the background mucosa</td>
<td>Infiltration of CD8-positive cytotoxic T cells</td>
<td>– Repression of tumor suppressor genes (e.g. <em>p14, p16</em> and <em>E-cadherin</em>)</td>
</tr>
<tr>
<td>Lower mortality rate</td>
<td></td>
<td>Alteration of host miRNA expression (e.g. suppression of miR-200)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Characteristic alterations of the host genome</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Frequent mutation of <em>PIK3CA, ARID1A</em> and <em>BCOR</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Rare mutation of <em>TP53</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Frequent amplification of <em>PD-L1</em> and <em>PD-L2</em></td>
</tr>
</tbody>
</table>

host-cell machineries (virus strategy). We then highlight somatic gene mutations of host cells, which might be prerequisite or accelerating factors for virus-driven oncogenesis in the stomach. This review only briefly mentions viral latent gene products; therefore, we refer readers to a more comprehensive review of this area [6]. As an overview of this review, table 1 summarizes the characteristics of EBVaGC.

Epidemiological, Clinical, and Pathological Features of EBVaGC

EBVaGC is defined as clonal growth of EBV-infected stomach epithelial cells. Practically, EBVaGC can be identified by in situ hybridization (ISH) of EBV-encoded small RNAs (EBER1/EBER2) on formalin-fixed paraffin-embedded sections of gastric carcinomas [7]. This is based on the fact that EBERs are abundantly produced in the nucleus of latently infected cells (10^6–7 copies per cell); nearly all cancer cells are positive for EBER-ISH, while adjacent nonneoplastic gastric epithelial cells and infiltrating lymphocytes are negative [8]. Thus, positive nuclear signals for EBER-ISH are regarded as the gold standard for defining EBVaGC.

Epidemiology

The frequency of EBV infection in gastric carcinoma ranges from 2 to 20%, with an average of ~10% [9]. EBVaGC is distributed worldwide with no endemic regions, although regional occurrence is well known in EBV-positive neoplasms, such as endemic Burkitt’s lymphoma in Africa and New Guinea, and nasopharyngeal carcinoma in China and Southeast Asia [10, 11]. Based on the Global Burden of Disease 2010 datasets, annual worldwide deaths from EBV-attributed malignancies are estimated at 69,081 cases of gastric carcinoma (EBVaGC), 63,118 cases of nasopharyngeal carcinoma, 7,917 cases of Hodgkin’s lymphoma and 2,251 cases of Burkitt’s lymphoma [12].

Clinical Features

According to a meta-analysis [13], the clinical features of EBVaGC include a predominance among male individuals and a predominant location in the proximal and middle regions of the stomach and in the remaining stomach after partial gastrectomy for gastric ulcer or gastric carcinoma (35.1% of total remnant stomach cancers; 95% CI 24.5–50.2%). Camargo et al. [14] recently reported that smoking is a risk factor for EBVaGC (odds ratio of 1.5; 95% CI 1.01–2.3). The frequency of *Helicobacter pylori* infection is controversial; some reports indicate less frequent *H. pylori* infection in EBVaGC [15, 16], while others indicate that the infection frequency is higher in EBVaGC than in other gastric cancers [17, 18]. However, the background mucosa in EBVaGC is characterized by severe atrophic gastritis with a paucity of intestinal metaplasia [17, 19].

The prognostic impact of EBV infection on gastric carcinoma is controversial. However, a recent meta-analysis of 4,599 gastric carcinoma cases (the largest to date), demonstrated that EBV positivity is associated with a reduced mortality rate after adjusting for the stage and other possible confounds (hazards ratio of 0.72; 95% CI 0.61–0.86) [20].

Pathological Characteristics

Although the predominant site of gastric carcinoma is the antrum, EBVaGC frequently occurs in the upper and middle portion of the stomach. According to our previous study, EBVaGC is located in the cardia (58%), body (33%) and antrum (9%) [21]. The gross appearance of EBVaGC is usually ulcerated or saucer-like, and the gastric wall is markedly thickened [7]. These characteristics can also be recognized by computerized tomography scanning [22]. EBV positivity does not correlate with the invasion depth.
regulated in mouse xenografts of human EBVaGC (Kanto and progression has not been determined. IL-1β is up-
the host immune response during tumor development 
however, the mechanism by which carcinoma cells evade 
observed in patients with EBVaGC.
Synchronous multiple gastric carcinomas are frequently 
stage, is a ‘lace pattern’
partially contribute to antitumor immunity 
and tumor cells 
might recruit numerous nonspecific lymphocytes to pre-
vent direct contact between EBV-specific cytotoxic T cells 
and tumor cells. IL-1β might recruit numerous nonspecific lymphocytes to pre-
vent direct contact between EBV-specific cytotoxic T cells 
and tumor cells.

Infiltrating lymphocytes in EBVaGC are predomin-
antly CD8-positive cytotoxic T cells [32] and at least 
partially contribute to antitumor immunity [33, 34]; however, the mechanism by which carcinoma cells evade 
the host immune response during tumor development 
and progression has not been determined. IL-1β is up-
regulated in mouse xenografts of human EBVaGC (KT tumor [35]) and in surgical specimens of EBVaGC. IL-1β 
might recruit numerous nonspecific lymphocytes to pre-
vent direct contact between EBV-specific cytotoxic T cells 
and tumor cells [36]. In addition, major histocompatibil-
ity complex genes and the genes that regulate chemokine 
activity are more frequently deregulated in EBVaGC tis-
sues relative to nontumor tissues [37].

**Virus-Driven Oncogenesis**

Before reviewing viral strategies to survive in the stom-
ach mucosa, we should mention the initial step of ‘EBV 
infection of epithelial cells’, the mechanism of which re-
 mains largely unknown. The mechanism of EBV epithe-
lial cell infection is different from that in B lymphocytes: 
gastric epithelial cells lack expression of major histoocom-
patibility complex class II and CD21, which work as re-
ceptors for EBV particles to enter B lymphocytes [38]. While viral protein gp42 interacts with major histocom-
patibility complex class II in B lymphocytes, another viral protein, gHgL, forms a complex with human αvβ5, αvβ6
or αvβ8 integrin, which triggers fusion between the viral 
particle and host epithelial cell [39]. Viral glycoprotein 
BMRF-2 also interacts with cellular integrin and mediates infection [40]. EBV infection of gastric carcinoma cell 
lines can be established with an approximately 800-fold-
higher efficiency by cell-to-cell contact using the EBV-
positive lymphocyte cell line, Akata, compared with di-
rect incubation with viral particles [41]. Furthermore, 
a virus released from B cells is 5-fold more infectious for 
epithelial cells than B cells [39]. It is possible that EBV is transferred from EBV-infected B lymphocytes to gastric 
epithelial cells when the virus is reactivated (from latent to replicative infection) in B lymphocytes at the stomach 
mucosa. EBER-positive epithelial cells are rarely observed on the surface of gastric mucosa, suggesting that most infection is abortive [42]. However, once epithelial cell in-
fec tion is established, the virus adopts the mechanisms described below for its survival (fig. 2).

**Virus Strategy 1**

Clonal EBV-DNA Is Maintained in EBVaGC

DNA in EBV viral particles is double stranded and lin-
ear, and becomes circular and episomal within infected 
cells. Varying numbers of terminal repeats (TRs) are ex-
cised from both ends of EBV DNA at the fusion of the linear DNA and, therefore, the number of TRs is different 
in each infected cell. Southern blot analysis of EBV-TRs, 
when applied to EBVaGC, demonstrates that EBV is 
monoclonal in each carcinoma of advanced stages [42, 
43], and monoclonal or oligo-clonal even in its intramu-
cosal stage [44]. The clonality of the viral genome suggests 
that the infection of EBV is an early event of carcinogen-
ess of EBVaGC. Although there has been no study which 
disclosed the significance of oligo-clonal infection of EBV 
in the early stage, it is possible that one of the clones be-
comes invasive and progresses to the advanced stage, re-
resulting in the monoclonality. Southern blot analysis also 
shows that the EBV DNA copy number per cell is variable 
in each case of EBVaGC, from several copies to more than 
100 [42]. Although the viral genome is sometimes inte-
grated into the host genome instead of forming epimases in lymphoma or nasopharyngeal carcinoma with EBV in-
fec tion [45], integration of the viral genome has not been 
reported in EBVaGC. Analysis of 71 cases of gastric car-
cinoma in The Cancer Genome Atlas (TCGA) database 
detected EBV transcripts in 4 cases but found no evidence

[9, 23], which is compatible with the assumption that EBV 
plays an essential role in the development of the carcino-
ma. Some studies demonstrated that the rate of lymph 
ode metastasis was lower in EBVaGC than other sub-
types of gastric cancer [24, 25], especially at the T1 stage. 
Synchronous multiple gastric carcinomas are frequently 
observed in patients with EBVaGC [26, 27].

There are two EBVaGC histological types, lymphoe-
epithelioma-like carcinoma, LELC, which is also known as 
gastric carcinoma with lymphoid stroma, GCLS (fig. 1), 
and conventional-type adenocarcinoma [28], although 
there is a morphological continuum between these types. 
Morphologically, most LELCs or GCLSs are EBVaGC 
[13]. Another feature of EBVaGC, especially in its early 
stage, is a ‘lace pattern’ [29], which shows irregularly 
anastomosing tubules and cords associated with moder-
ate-to-dense lymphocytic infiltration and results in a 
lace-like or reticular pattern at low magnification.

With regard to cell differentiation, EBVaGC displays 
unique features; nearly half of cases are positive for gas-
tric-type mucin (MUC5AC and MUC6), while the other 
half are negative for gastric-type mucin or intestinal-type 
markers (MUC2 and CD10). Most EBVaGCs express gas-
tric-type claudin, CLDN18, but not the intestinal type, 
CLDN3 [30, 31].

Infiltrating lymphocytes in EBVaGC are predomi-
nantly CD8-positive cytotoxic T cells [32] and at least 
partially contribute to antitumor immunity [33, 34]; however, the mechanism by which carcinoma cells evade 
the host immune response during tumor development 
and progression has not been determined. IL-1β is up-
regulated in mouse xenografts of human EBVaGC (KT tumor [35]) and in surgical specimens of EBVaGC. IL-1β 
might recruit numerous nonspecific lymphocytes to pre-
vent direct contact between EBV-specific cytotoxic T cells 
and tumor cells [36]. In addition, major histocompatibil-
ity complex genes and the genes that regulate chemokine 
activity are more frequently deregulated in EBVaGC tis-
sues relative to nontumor tissues [37].

**Virus-Driven Oncogenesis**

Before reviewing viral strategies to survive in the stom-
ach mucosa, we should mention the initial step of ‘EBV 
infection of epithelial cells’, the mechanism of which re-
 mains largely unknown. The mechanism of EBV epithe-
lial cell infection is different from that in B lymphocytes: 
gastric epithelial cells lack expression of major histoocom-
patibility complex class II and CD21, which work as re-
of viral genome integration into the host genome [46]. Another study of whole-genome sequencing including 4 cases of EBVaGC also identified no chromosomal integration of the viral genome [47].

The earliest events in the development of EBVaGC have not yet been clarified, but one or several infected cells in the stomach mucosa containing original copies of EBV DNA ultimately transform to start clonal growth. Viral nuclear protein, EBNA1, is a multifunctional protein that is necessary for maintaining viral infection in host cells by contributing to replication and mitotic segregation of the viral genome. EBNA1 recruits a cellular origin-recognition complex, which is necessary for DNA replication, to OriP of the viral DNA. Using the cellular machinery, viral replication is initiated during the host cell S phase. In addition, EBNA1 tethers viral episomes to the host chromosomes, which enables propagation of the same copy number of viral genomes to the daughter cells at mitosis [48].

**Virus Strategy 2**

Epigenetic Silencing of Viral Genes and Host Genes

Epigenetic regulation of both viral and host genes is important in EBV-associated neoplasms. Histone modifications and chromatin organization are just beginning to be investigated [49]; therefore, we will focus on DNA methylation in this review, which has been relatively well studied. The viral DNA, including transcription initiation sites for lytic genes, is densely methylated in latently infected cells [50]. When viral immediate early protein and transcription factor Zta (ZEBRA, Z, BZLF1) is expressed in response to reactivation agents, it selectively binds to methylated DNA of viral lytic promoters, causing the infected cell to transit from latency to lytic infection [51]. Latent EBV infection is classified into three forms by the expression patterns of latent genes. EBER-1 and 2, EBV-determined nuclear antigen (EBNA)-1, Bam-HI-A region rightward transcripts (BARTs), and BART microRNAs (miRNAs; discussed later) are expressed in all latency types. In latency type II, latent membrane protein (LMP)-1, 2A or 2B are also expressed, and latency type III includes all of these latent genes along with EBNA-2, 3A, 3B, 3C and LP. EBVaGC belongs to latency type I or II, in which EBERs, EBNA-1, BART’s and BART miRNAs are expressed, and approximately half of EBVaGC cases express LMP-2A. In determining the latency, CpG methylation of latent gene promoters also plays a central role, although it is not known how different methylation patterns are established [1]. It is surprising that DNA methylation also occurs at high frequencies in CpG islands of the promotor region of host genes in EBVaGC [52]. Various tumor suppressor genes, such as p14, p16 and E-cadherin, are suppressed through promoter methylation in EBVaGC [53–55]. Furthermore, this process is not random; for example, MLH1, which is methylated in microsatellite unstable gastric carcinoma, is not methylated in EBVaGC [56, 57]. Recently,
we performed a genome-wide comprehensive methylation analysis of gastric carcinoma, and identified three distinct epigenotypes (EBV−/low methylation, EBV+/high methylation, and EBV+/high methylation). In EBV+/high methylation gastric carcinoma, genes methylated in EBV−/high methylation gastric carcinoma and EBVaGC-specific genes are both extensively methylated. However, genes methylated in EBV−/high methylation gastric carcinoma were mainly the target of a polycomb recessive complex, while genes methylated specifically in EBVaGC were not. These observations suggest a unique or additional mechanism of DNA methylation in EBVaGC. Furthermore, using MKN7, a gastric cancer cell line with low methylation, we showed that global methylation was induced in EBVaGC-specific genes and in genes methylated in the EBV−/high methylation epigenotype by EBV infection [2, 3]. Interestingly, a study using oral keratinocytes revealed that DNA methylation of the host genome was induced by EBV infection and that some methylation was retained even after the virus was lost from the infected cells [58].

The exact mechanisms of EBV-driven hypermethylation, described above, remain unknown. We observed that the viral latent protein LMP2A induced 5-fold overexpression of DNMT1 through phosphorylation of STAT3, leading to methylation of the PTEN promoter in infected gastric cancer cell lines, although this pathway could only partially explain the global CpG-island methylation of EBVaGC [59]. Nevertheless, using an experimental EBV infection system, viral DNA methylation was shown to precede the methylation of host DNA by 1 week [Matsusaka et al., in preparation]. Therefore, the methylation of viral genes might be caused by a host defense mechanism against foreign DNA to suppress the expression of viral genes. However, this might result in other outcomes – repressing viral latent gene expression might benefit EBV by allowing it to evade a host’s immune response [60]. Excessive methylation also leads to the repression of tumor suppressor genes, as described above, which promotes the survival of infected cells.

Virus Strategy 3
Viral and Host microRNAs
miRNAs are small noncoding RNA molecules ~22 nucleotides in length encoded in the introns or exons of genes. miRNA precursors are processed from these transcripts and subsequently processed by Drosha and Dicer into mature forms. Mature miRNAs interact with the 3′-untranslated regions of target mRNAs and repress their translation [61]. EBV also encodes its own miRNAs in its genome. There are 25 miRNA precursors and 44 mature EBV miRNAs in EBV. Three of the miRNA precursors are derived from the BHRF1 cluster and the remaining 22 are from the BART cluster. BHRF1 expression is restricted to latency III, and only BART miRNAs are expressed in EBVaGC, or in other EBV-associated neoplasms of latency I and II [62–64]. We recently profiled the expression of the 44 known EBV miRNAs in tissue samples from patients with EBVaGC; ebv-miR-BART1–3p, 2–5p, 3, 4, 5, 7, 9, 10–3p, 17–5p, and 18–5p were expressed at relatively high levels. In silico analysis revealed that the target genes of these EBV miRNAs had functions associated with cancer-related pathways, especially the regulation of apoptosis. Apoptosis was in fact reduced in tissue samples of EBVaGC, and the gastric carcinoma cell lines infected with EBV exhibited downregulation of the proapoptotic protein Bid (the BH3-interacting domain death agonist), a member of the Bcl-2 family. Subsequent experiments demonstrated that ebv-miR-BART4-5p regulates Bid expression in EBVaGC [65]. Cai et al. [66] recently demonstrated in nasopharyngeal carcinoma that ebv-miR-BART7-3p, one of the most abundant viral miRNAs in EBVaGC, targets the tumor suppressor gene PTEN, and promotes epithelial-to-mesenchymal transition and tumor metastasis.

Human cellular miRNA expression profiles are also altered by EBV infection. We previously reported that two cellular miRNAs, hsa-miR-200a and hsa-miR-200b, were downregulated in EBVaGC, both in tissue samples and cell lines. These miRNAs target the transcription repressors ZEB1 and ZEB2, which regulate E-cadherin expression. Downregulation of these miRNAs ultimately reduced E-cadherin expression and triggered epithelial-to-mesenchymal transition. The EBV latent genes BARF0, EBNA-1 and EBERs cooperatively suppressed hsa-miR-200a and 200b expression in cell lines, while reduced ZEB1, ZEB2 and E-cadherin expression was significant only in EBER-transfected cells [67]. EBV infection causes a decrease in the levels of tumor suppressor miRNAs (antioncomirs) such as the let-7 family [64].

The role of miRNAs is not only intracellular regulation of gene expression, but also intercellular communication and modification of the tumor microenvironment [68]. Exosomes, small vesicles with lipid bilayer membranes, are derived from endoplasmic multivesicular bodies and carry proteins and RNAs from cell to cell. Pegtel et al. [69] demonstrated that miRNAs secreted by EBV-infected cells are transferred to and act in uninfected recipient cells. They showed by coculture of EBV-infected B cells

EBV-Associated Gastric Carcinoma
Pathobiology 2015;82:212–223
DOI 10.1159/000434683
217
Downloaded by: [54.70.40.11] - 10/6/2017 8:49:51 PM
that EBV-miRNAs accumulated in noninfected, neighboring monocyte-derived dendritic cells – MoDCs – through exosome transfer. The miRNAs, internalized in MoDCs, then downregulated their target molecules [70]. The significance of secreted miRNAs in EBVaGC is not clear at present, but some studies suggest functions of exosomes, which are derived from EBV-negative gastric cancer. For example, exosomes derived from gastric cancer cell lines induced mesenchymal stem cells to differentiate into carcinoma-associated fibroblasts [71], and induced apoptosis in Jurkat T cells [72]. Thus, it is possible that viral miRNAs in exosomes also play an important role in modulating the tumor microenvironment in EBVaGC. Furthermore, exosomal miRNAs are relatively stable in circulating blood and have been identified in the circulating blood of nasopharyngeal carcinoma patients. They may be useful as biomarkers of the diagnosis of EBVaGC [73–75].

**Molecular Abnormalities Demonstrated by ‘Omics’ Studies**

The TCGA Research Network recently reported the results of a comprehensive molecular evaluation of 295 gastric carcinomas according to different modalities, such as genomic alterations, gene expression profiling and proteomic analysis. They proposed a novel molecular classification which divides gastric carcinomas into four types: EBVaGC, microsatellite unstable tumors, genomically stable tumors and tumors with chromosomal instability. The study confirmed characteristic hypermethylation of DNA, and further identified recurrent mutations of ARID1A (55%) and PIK3CA (80%) in EBVaGC. Other frequent mutations included BCOR (23%), but TP53 mutation was extremely rare. Chromosomal abnormality is not common in EBVaGC, but amplification of JAK2, PD-L1 and PD-L2 were frequent in EBVaGC [5].

**ARID1A**

ARID1A is a subunit of the SWI/SNF chromatin remodeling complex and is thought to have a tumor suppression role by regulating chromatin structure and gene expression [76]. ARID1A is frequently mutated in microsatellite unstable gastric carcinoma as well as in EBVaGC [77, 78]. Mutation of ARID1A was first reported in ovarian clear cell and endometrioid adenocarcinoma [79, 80]. Subsequently, recurrent mutations of ARID1A have been reported in other carcinomas, such as colorectal [81], hepatoellular [82–85] and bladder carcinoma [86]. There are no hot spots for ARID1A mutations and most are missense or truncation mutations, which result in lost or weak expression of ARID1A protein [77, 79]. A high frequency of ARID1A mutation in microsatellite unstable gastric carcinoma can be explained by deficiency of the mismatch repair system because most of the mutations are indels, which is common in tumors with microsatellite instability [77, 87]. However, it is unknown why mutation of ARID1A is more frequent in EBVaGC compared with other gastric carcinomas.

We investigated the loss of ARID1A expression by applying immunohistochemistry to a large series of gastric carcinoma tissue microarrays. Loss of ARID1A was frequently observed in EBVaGC and MLH1-negative gastric carcinoma (corresponding to the microsatellite unstable type [88–90], as described above). Characteristic to EBVaGC is homogeneous loss of ARID1A within the tumor (all-or-none-type expression) independent of the invasion depth, indicating that loss of ARID1A is an early event in EBVaGC carcinogenesis [21]. According to a deep sequencing study of nonneoplastic gastric mucosa [91], ARID1A mutations were present in chronically inflamed and H. pylori-infected gastric mucosa, although the copy number of the mutated gene was low. These observations suggest that the mutation event precedes EBV infection. Mutations in ARID1A, leading to altered chromatin structures, might facilitate viral entry to the nucleus or manipulation of methylation mechanisms [92, 93].

**PIK3CA**

PIK3CA is an important regulator of the PI3K/Akt pathway. Mutations in PIK3CA are frequent in cancers of various organs, such as colon, breast, endometrium and ovary. These mutations are mostly hot-spot mutations located in exon 9 (E542K and E545K) and exon 20 (H1047R) [94, 95]. It is of interest that the mutation sites in PIK3CA are distributed widely in EBVaGC [5] with only 28% of mutations in hot spots. A considerable number of these non-hot-spot mutations, however, show moderate-to-weak transformation activity and/or lipid phosphorylation activity [96]. Similarly to ARID1A mutation, it is possible that PIK3CA mutation precedes EBV infection, which then augments functions of mutated PIK3CA to activate the PI3K/Akt pathway. The viral protein LMP2A is known to upregulate PI3K/Akt pathways [97], and EBV-induced DNA methylation of PTEN and INPP4B (inhibitors of the pathway) could contribute to this process [59, 98]. The PI3K/Akt pathway is involved in many
biological processes [97]; therefore, the significance of its activation through non-hot-spot mutations in early carcinogenesis of EBVaGC is far from clear. However, EBV infection in immortalized nasopharyngeal epithelial cells showed sustained activation of pAkt under nutrient deficiency, which contributed to their survival [99]. Furthermore, the coexistence of ARID1A and PIK3CA mutations is frequently observed in ovarian clear cell carcinoma and in some foci of endometriosis, especially atypical endometriosis [100, 101]. Chandler et al. [102] recently demonstrated that both mutations cooperate to promote tumor growth through sustained IL-6 overproduction in the ovarian surface epithelium of a mutant mouse model of ovarian clear cell carcinoma. Therefore, further studies are necessary to clarify how EBV uses the consequences of these mutations in EBVaGC.

The hypothetical crosstalk between EBV and PIK3CA mutations, however, should be cautiously considered. According to other sequencing studies of gastric carcinoma, the frequencies of PIK3CA mutation vary considerably in EBVaGC [4, 77, 103, 104]. The variable frequencies might be due to the tumor content or the methods used in the studies.

**BCOR**

The Bcl-6 corepressor antiapoptotic protein BCOR [105] is frequently mutated in acute leukemia [106, 107] and medulloblastoma [108]. Tiberi et al. [109] recently demonstrated that BCL6 represses GLI1 and GLI2, effectors of the SHH pathway, through recruitment of BCOR and SIRT1 deacetylase. Because the targets of the BCL6/BCOR/SIRT1 complex depend on the cellular context, further studies are necessary to clarify the significance of this mutation in EBVaGC.

**Amplification of PD-L1 and PD-L2**

As mentioned previously, comprehensive analysis of gastric carcinoma revealed amplification of PD-L1 and PD-L2 in EBVaGC [5]. The finding is very interesting because these genes are thought to play a role in evasion from tumor immunity. Both PD-L1 and PD-L2 are ligands of PD-1 (programmed death-1), which is a coreceptor of mature CD8+ and CD8+ T cells, and activation of the PD-1 pathway induces immune tolerance [110]. Overexpression of PD-L1 and subsequent evasion from tumor immunity is a known process for cancer progression, and carcinomas with PD-L1 expression are more aggressive and associated with a worse prognosis in renal cell carcinoma [111]. Gastric carcinoma with PD-L1 expression is also associated with an advanced tumor stage and worse prognosis [112, 113]. Because EBVaGC has markedly dense CD8-positive lymphocytic infiltration in the tumor cell nest and tumor stroma [32], escape from the T cell immune system is essential for tumor progression. Blockade of the PD-L1/PD-1 pathway with anti-PD-L1 antibody showed an anticancer effect in some clinical trials [114–116]. Therapy targeting PD-L1 might also be effective in EBVaGC, and further study of PD-L1 in EBVaGC is necessary.

**Conclusions**

EBVaGC is a distinct gastric carcinoma, not only in its unique epidemiological, clinical and histological characteristics, but also in its molecular mechanisms. Viruses use cellular machineries for their survival, for example to propagate the monoclonal viral genome, to epigenetically silence viral and host genes, and to control the infected cell’s behavior and microenvironment. In addition, recent comprehensive molecular analyses (‘omics’ studies) have demonstrated frequent alterations of the host genome (frequent mutation of PIK3CA, ARID1A and BCOR, and amplification of PD-L1 and PD-L2). High-frequency mutations, such as in PIK3CA and ARID1A, might be a prerequisite for the development of EBVaGC.
In EBV-positive Burkitt’s lymphoma, EBV is considered to rescue B cells with Ig-myc translocation, which are otherwise excluded by apoptosis [117]. Lower-frequency mutations, such as in BCOR or amplification of PD-L1 and PD-L2, might contribute to cancer progression and immune evasion (fig. 3). Further studies are necessary to clarify this coordination of virus and host cell mutations to enable targeting of therapeutic candidates against this particular subtype of gastric carcinoma.

Acknowledgments

This work was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (JSPS; Grant No. 26860232 to H.A. and 26253021 to M.F.), and from the Core Research for Evolutionary Science and Technology (CREST) program from the Japan Science and Technology Agency to A.K. and M.F.

References

EBV-Associated Gastric Carcinoma

Pathobiology 2015;82:212–223

DOI: 10.1159/000434683

221


EBV-Associated Gastric Carcinoma

Pathobiology 2015;82:212–223
DOI: 10.1159/000434683