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Review article

Epstein-Barr virus and gastric carcinoma

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Abstract:
Epstein-Barr virus (EBV)-associated gastric carcinoma (EBVaGC), which accounts for most lymphoepithelioma-like gastric carcinoma and 10% or less of ordinary gastric carcinoma, is found worldwide and is the most common EBV-associated neoplasm. The pathologic features of EBVaGC are male predominance, occurrence primarily in the proximal stomach, multiplicity, moderately or poorly differentiated histological type, and association with lymphocytic infiltration. Virologically, EBV in EBVaGC is monoclonal or oligoclonal in the intramucosal or early invasive stage, and invariably monoclonal in advanced carcinomas. Latency type of gene expression in EBV is the same as that in Burkitt lymphoma (latency type I). Thus, EBVaGC is a unique type of gastric carcinoma, which is derived from a single or a few EBV-infected epithelial cells. However, there are still some unanswered questions, such as the mechanism of EBV infection of epithelial cells, the primary target in the gastric epithelium, the molecular events underlying EBVaGC, and the interaction of EBV with cytokine genes in cancer cells, as well as the predisposing factors for EBVaGC. To develop an experimental model, we recently established a transplantable EBVaGC — in severe combined immunodeficiency (SCID) mice — which retains the original EBV and the same pattern of latency gene expression as the original carcinoma. The development of a new therapeutic approach using this model, such as a gene therapy specific to EBV-associated neoplasm, will make EBVaGC not only a pathologically but also a clinically distinct gastric carcinoma entity.

Key words: Epstein-Barr virus, gastric carcinoma

Introduction

Epstein-Barr virus, a gammaherpes virus, is the first virus that was identified in a human neoplastic cell [1]. More than 90% of the world population is infected with EBV before adolescence, and it has been shown that some limited populations develop EBV-associated malignancy in an endemic manner, such as Burkitt lymphoma in equatorial Africa [2] and nasopharyngeal carcinoma (NPC) in Southern China [3]. However, recent advances in molecular techniques have demonstrated an unexpectedly wide variety of neoplasms associated with EBV [4,5] (Table 1), among which EBV-associated gastric carcinoma (EBVaGC) is the most common, with a worldwide distribution [6–9]. In Japan, for example, 5000 patients are estimated to develop gastric carcinoma annually in association with EBV.

Gastric carcinoma, whether EBVaGC or not, is a very common carcinoma in Japan, South America, and Eastern Europe [10]. *Helicobactor pylori* has been implicated as a causative agent in most gastric carcinomas, but its effect may be indirect, through causing sustained injury to the mucosa, resulting in atrophic gastritis, intestinal metaplasia, and precancerous lesions [11,12]. On the other hand, while EBVaGC accounts for 10% or less of gastric carcinoma, EBV seems to play a direct role in the development of this carcinoma [8,9], or at least the carcinoma cells are tagged with clonal EBV, which makes EBVaGC a distinct entity among gastric carcinomas.

In 1990, Burke et al. [13] first demonstrated the relationship between EBV and gastric carcinoma, by identifying EBV-DNA by polymerase chain reaction (PCR) in a lymphoepithelioma-like carcinoma of the stomach

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Table 1. Epstein-Barr virus (EBV)-associated neoplasms [4,5]

Carcinomas	**Nasopharyngeal carcinoma**
	Lymphoepithelioma-like carcinoma
	Stomach
	Parotid gland
	Thymus
	Lung
	Gastric carcinoma
Lymphoproliferative disorders	**Endemic Burkitt lymphoma**
	Opportunistic lymphoma
	Pyothorax-associated lymphoma
	Nasal Natural killer-cell lymphoma
	Chronic active EBV infection
	Hodgkin's disease
	B- or T-cell lymphoma
Other	**Spindle-cell tumor in immunocompromised hosts**

Items in bold type: association with EBV observed in almost 100% of the neoplasms

(Fig. 1A, B). Lymphoepithelioma is a descriptive term for the histologic features of NPC, a poorly differentiated carcinoma accompanied by prominent infiltration of lymphocytes. Gastric carcinoma with similar histologic features has also been termed "gastric carcinoma with lymphoid stroma", which shows a favorable prognosis compared with that of other poorly differentiated adenocarcinomas [14]. Based on the results of a highly sensitive in-situ hybridization (ISH) method targeting EBV-encoded small RNA (EBER) [15], most lymphoepithelioma-like gastric carcinomas are now considered to be associated with EBV [16–19] (Fig. 1E). However, it is interesting that apart from the nasopharynx, parotid gland, and stomach, a close association between histology and EBV etiology is occasionally observed in thymic tumors and lung carcinomas, but not in tumors of the breast, uterus, or other intestinal tract tissues [20,21]. In the stomach, the association with EBV is not limited to lymphoepithelioma-like carcinoma, but is also observed in some gastric carcinomas with ordinary histology (Fig. 1C, D, F).

General aspects of EBV infection

As occurs with other herpes viruses, individuals infected with EBV become life-long carriers of the virus. In most nosocomial infections, EBV infects humans via salivary contact (Fig. 2). There are two major target cell types for EBV infection: B lymphocytes, in which the infection is largely nonproductive or latent, and epithelial cells of the oropharynx, in which viral replication occurs. It has generally been thought that the oropharyngeal epithelium is the main site for the intermittent production of infectious virus. B lymphocytes are then infected by the virus by circulating within the mucosa, and only a small number of EBV-infected B lymphocytes, in which EBV becomes latent, can escape the attack of EBV-specific cytotoxic T cells (CTL). However, these B lymphocytes deliver EBV infection to remote organs, the microenvironment of which favors reactivation of latent virus, resulting in the subsequent infection of epithelial cells [22,23].

EBV is an envelope icosahedral virus containing a 172-kilo-base pair double-stranded linear DNA (Fig. 3). Upon infection, the viral DNA is transported into the nucleus, where it exists predominantly as an extrachromosomal circular molecule (episome). The EBV genome contains over 100 open reading frames, which potentially encode as many peptides. However, in the latent viral state, such as in B lymphocytes of healthy carriers or in neoplastic cells of EBV-associated neoplasms, the viral genes that can be expressed are restricted to a family of EBV nuclear antigens (EBNA1, 2, 3A, 3B, 3C and EBNA leader proteins), three latent membrane proteins (LMP1, LMP2A, and 2B), two small RNAs (EBER1 and 2), and a group of transcripts in the *Bam*HI A region of the EBV genome [4,22]. EBNA1 is indispensable for the maintenance of latent infection. EBNA2 transactivates viral and cellular genes, and is a key determinant of lymphocyte transformation. LMP1 has transforming effects in rodent fibroblastic cell lines, and expression of LMP1 in the skin of transgenic mice results in epidermal hypertrophy. EBERs are nonpolyadenylated, nontranslated small RNAs, and are produced in large amounts: 10^{6-7} copies per infected cell. Based on this finding, ISH has been widely used to identify EBER in epidemiological and pathological studies to demonstrate latently infected cells in routine formalin-fixed and paraffin-embedded sections.

Three types of latency have been described for lymphoid cell lines, based on the variable expression of the latent gene products, and they are used to classify EBV-associated neoplasms, as shown in Table 2 [4,22]. In latency type I, antigen expression is restricted to EBNA1, which is driven by a promoter in the *Bam* H I-Q fragment of the genome (Qp) (Fig. 3). In latency type II, EBNA-1 is also under the control of the Qp promoter, but there is detectable transcription of LMP1 and 2 by LMP promoters. In latency type III, all of the EBNAs and all of the LMPs are expressed. The transcripts for EBNAs are driven by the the promoter on *Bam* H I-W or -C fragment (Wp or Cp, respectively). Clarification of EBV gene expression in EBVaGC is an important issue for understanding not only the oncogenic potential of EBV in various tumors, but also the immunobiology of the tumor, since EBV-specific CTL can recognize all EBV-coded latent-phase proteins, except for EBNA1.

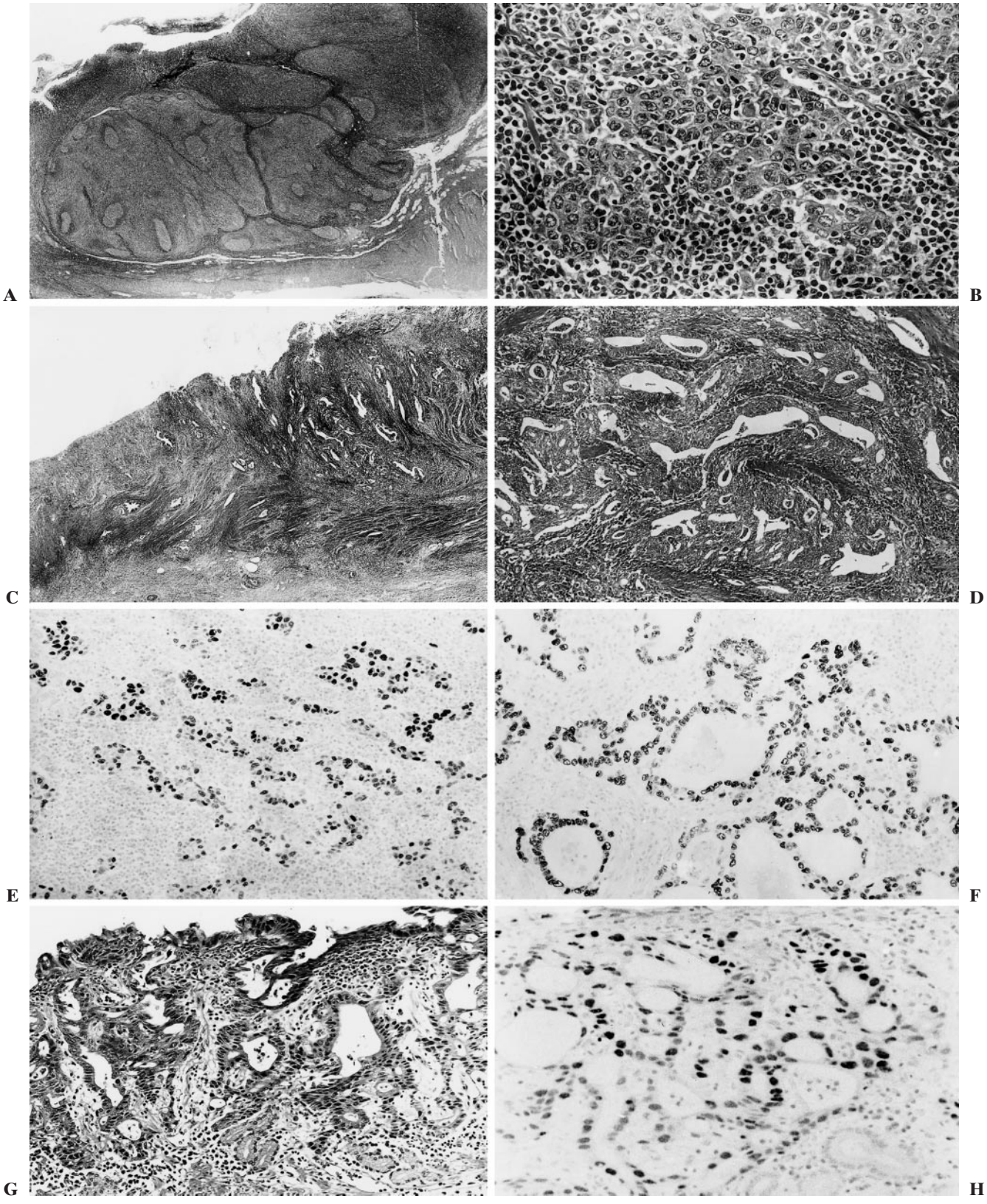

Fig. 1A–H. Histological features of Epstein-Barr virus (EBV)-associated gastric carcinoma (EBVaGC). (**A**, **B**) Lymphoepithelioma-like gastric carcinoma is accompanied by diffuse infiltration of lymphocytes and lymphoid follicles. (**C**, **D**) Gastric carcinoma with ordinary histology consists of tubular structures with varying degrees of lymphocyte infiltration. (**E**, **F**) By in-situ hybrydization (ISH) targeting EBV-encoded small RNA (EBER), a positive signal is demonstrated in the nuclei of carcinoma cells of both types of gastric carcinoma. (**G**, **H**) In the intramucosal stage of EBVaGC, the carcinoma is likely to have a lacy pattern in the proper mucosa. Note the preserved pyloric glands beneath the tubular structures of EBVaGC. **E**, **F**, and **H**: EBER-ISH

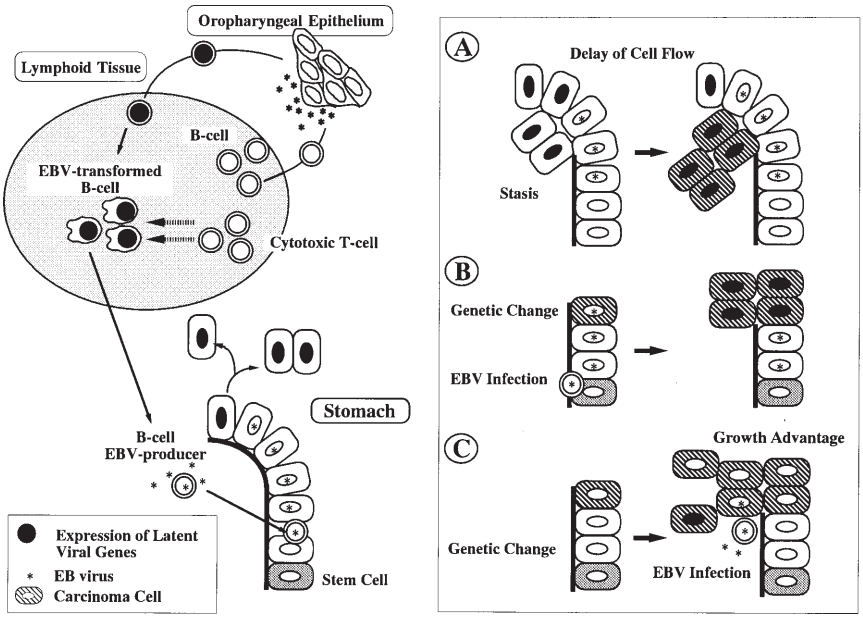

Fig. 2. EBV infection in epithelial cells of the stomach and a possible mechanism for EBV infection in the carcinogenesis of EBV

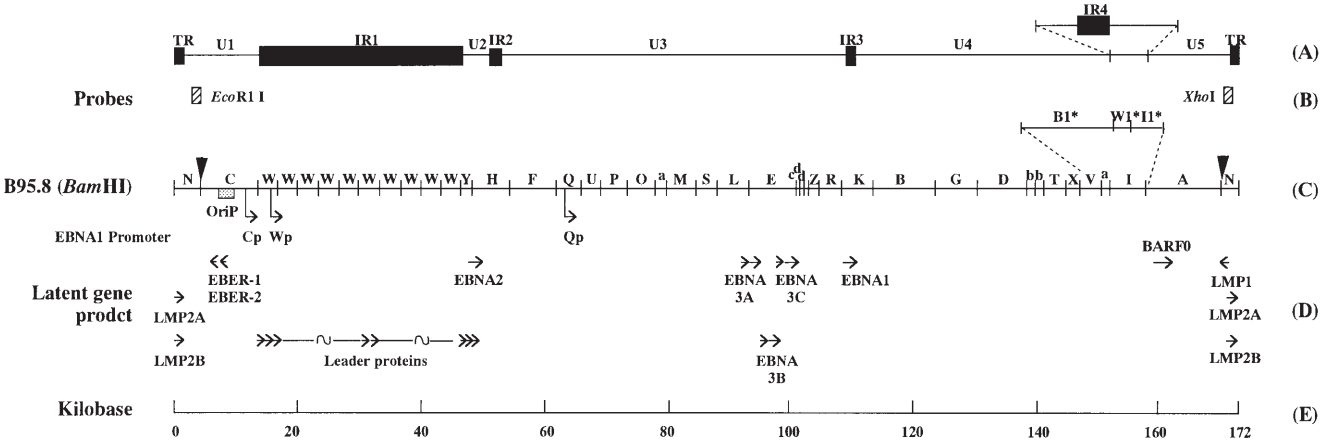

Fig. 3. Genomic map of EBV [22]. *A* Organization diagram of the B95-8 genome. The deletion in the *Bam* H I I region relative to other EBV genotypes is indicated. *TR*, Terminal repeat; *U1–5*, unique sequences 1–5; IR 1–4, internal repeats 1–4. *B* Probes used for the clonal analyses, the principles of which are shown in *Fig. 4. C* Positions of the B95-8 *Bam* H I restriction fragments. *Arrowheads* indicate the restriction enzyme sites presented in *Fig. 4. D* Positions of the origin of plasmid replication (oriP), EBNA (EBV nuclear antigen) promoters (Cp, Wp, and Qp) and selected open reading frames for latent gene products. *E* bp coordinates of the B95-8 genome, expressed in kb

Table 2. EBV gene expression in three latency types of EBV-associated neoplasms

Latency type	EBER	Promoter	EBNA	LMP	Cell type in human neoplasm
I	1, 2	Qp	1		Burkitt lymphoma, EBVaGC
II	1, 2	Qp	1	1, 2A, 2B	NPC, Hodgkin's disease
III	1, 2	Wp/Cp	1, 2, 3s, LP	1, 2A, 2B	Opportunistic lymphoma, PAL

For explanation of latency type, see text
EBVaGC, Epstein-Barr virus-associated gastric carcinoma; EBER, EBV-encoded small RNA; EBNA, EBV nuclear antigen; LMP, Latent membrane protein, NPC, nasopharyngeal carcinoma; PAL, pyothorax-associated lymphoma

Table 3. Frequency of EBV involvement in gastric carcinoma

Report	Country	Frequency of EBV involvement (%)		LEC in EBVaGC (%)
		In GC overall	In LEC	
Shibata and Weiss [6]	USA	22/138 (15.9)	ND	3/22 (13.6)
Tokunaga et al. [7]	Japan	69/999 (6.9)	8/9 (88.9)	8/69 (11.6)
Leoncini et al. [24][a]	Italy	3/65 (4.6)	0	0/3 (0)
Rowlands et al. [25]	UK, Japan	9/174 (5.2)	6/6 (100)	6/9 (66.7)
Fukayama et al. [8]	Japan	8/78 (11.1)	2/2 (100)	2/8 (25.0)
Nakamura et al. [17]	Japan	4/42 (9.5)	82/99 (82.8)	ND
Ott et al. [26]	Germany	7/39 (17.9)	4/4 (100)	3/7 (42.9)
Yuen et al. [27]	China	7/74 (9.5)	1/3 (33.3)	1/7 (14.3)
Harn et al. [28]	Taiwan	6/55 (10.9)	ND	1/6 (16.7)
Shin et al. [29]	Korea	12/89 (13.5)	ND	10/12 (83.3)
Galetsky et al. [30]	Russia	18/206 (8.7)	0	0/18 (0)

ND, Not described; GC, gastric cancer; LEC, lymphoepithelioma-like gastric carcinoma
[a] This study used DNA in-situ hybridization (ISH) with *Bam*HI W probes; the others used EBER-ISH

Pathology of EBVaGC

Based on the application of EBER-ISH to formalin-fixed and paraffin-embedded tissues, EBVaGC has been reported to account for 5%–18% of gastric carcinomas in various countries [6–9,24–30] (Table 3). EBVaGC is unlikely to be an emerging neoplasm, since the proportion of EBV involvement in gastric carcinoma seemed to be constant in series in 1948–1950 and in the 1990s in Los Angeles [31]. Tokunaga et al. [32], examined EBV involement in gastric carcinoma in the populations in many Japanese cities and found that the proportion of EBVaGC in gastric carcinoma tended to be reciprocal to the mortality rate, with 3.1% EBV involvement and a mortality rate of $57.2/10^5$ in Niigata and 10.3% EBV involvement and a mortality rate of $15.8/10^5$ in Okinawa. Shibata [31] reported that the frequency of EBVaGC involvement in gastric carcinoma was higher among Japanese living in Hawaii than in Japan. Although the differences in EBVaGC frequency between geographic regions have not yet been clearly explained, the incidence of EBVaGC in the general population may be constant between regions and may become relatively more important in gastric carcinoma as other risk factors are reduced.

EBVaGC is more frequent in tumors from males. The site of EBVaGC within the stomach is another characteristic: it occurs predominantly in the proximal stomach, particularly in the gastric cardia [7,8]. A high frequency of EBVaGC was observed in de-novo carcinoma in the remnant stomach [33]. However, in our study, EBV involvement in the remnant stomach was not significantly different from that in the cardia. On the other hand, in patients in whom the first carcinoma was EBVaGC, the second carcinoma in the remnant stomach, which occurred within 10 years, was also EBVaGC in six of nine cases (Kaizaki et al., manuscript in preparation). Matsunou et al. [19] reported that EBVaGCs occurred much more frequently than would be expected if the development of EBVaGC was assumed to be independent: nine of ten carcinoma lesions in four cases of synchronous multiple carcinomas and all five carcinoma lesions in two cases of metachronous carcinomas were EBVaGC. These findings suggest that the non-neoplastic mucosa of the proximal stomach bearing EBVaGC has been conditioned to develop EBVaGC (field cancerization).

EBVaGC is observed in gastric carcinomas at all depths of invasion, although the proportion of EBVaGC in intramucosal carcinoma is relatively or significantly lower than that in invasive carcinomas (4.4% vs 7.4% at Kagoshima [7] and 5.7% vs 14.5% at our hospital). The lower rate of EBVaGC in intramucosal carcinoma may reflect the presence of EBV-negative and less aggressive neoplasms in intramucosal carcinoma in our series. This possibility has been pointed out in a comparative study of the criteria for gastric carcinoma used by Japanese and Western pathologists [34].

As for the histology of the carcinoma (Fig. 1), most lymphoepithelioma-like gastric carcinomas are associated with EBV. The proportion of this particular type in EBVaGC varies considerably, from 0 to 80% (25% in our study; Table 3), according to the strictness of the criteria. Nevertheless, EBVaGC with ordinary histology has certain characteristic morphological features: moderately differentiated tubular and poorly differentiated solid types are predominant, while papillary and scirrhous types are extremely rare. This indicates that EBVaGC may not have the same carcinogenic process

as intestinal or diffuse type gastric carcinoma. It is also interesting that EBVaGC in its intramucosal stage is likely to exhibit a specific histological pattern (Fig. 1G, H): abortive tubular structures occupy the middle of the mucosa without destroying the mucosal architecture (lacy pattern) [35].

The clinical outcome of EBVaGC has not been studied in detail. It appeared to be favorable when metastatic carcinomas were evaluated [19], but the prognosis of patients with advanced EBVaGC was not significantly different from that of patients with EBV-negative carcinomas (our unpublished observation). Nakamura et al. [17] demonstrated that the prognosis of patients with lymphoepithelioma-like gastric carcinoma was not affected by whether the carcinoma was associated with EBV.

Virology of EBVaGC

EBV is a double-stranded DNA virus with repetitive 500-bp structures at both ends (terminal repeat, TR), and the viral particles contains a linear form of EBV DNA (Fig. 3). After EBV enters the nuclei of the infected cells, it becomes circular by fusion of its ends (Fig. 4). The specific structure of both ends of EBV DNA has been used to provide evidence of clonality of EBV-associated neoplasms, to indicate viral integration, and to suggest the state of viral activation in cells; i.e., replicating (linear configuration) versus latent (episomal circular forms) [36,37] The principle of clonal analysis is as follows (Fig. 4): Due to both the number of TR at the end of the linear EBV genome and the extent of their overlapping during episome formation, each new circularization event leads to a different-size TR fragment. In latently infected cells, the replication of episomes is regulated by and parallels host-cell proliferation, so that the genomic structure and the copy numbers of the episomes remain stable in the infected cell and its progeny. Thus, circular, linear, or integrated EBV genomes can be distinguished by analyzing the viral genomic structure with probes to the unique DNA sequences at either end of the EBV genome (Fig. 4). For example, enzymatic digestion of episomal DNA, with an enzyme such as *Bam* H I that spares the TR sequences, produces a single fused terminal fragment in monoclonal cells (Fig. 4A). Permissively infected cells have a ladder array of small terminal fragments representing linear viral DNA (Fig. 4B). If the virus is integrated through the TR, distinct restriction fragments representing viral/cellular junction fragments are detected (Fig. 4C). Using this approach, EBV is invariably monoclonal and episomal in EBVaGC in the advanced stage and in metastasis [8,9,26]. We recently observed that EBV was mono- or oligoclonal in intramucosal or early invasive carcinomas (manuscript in preparation). Therefore, EBV involvement in EBVaGC may precede the clonal growth of carcinoma cells, or at least may take place at its earliest stage.

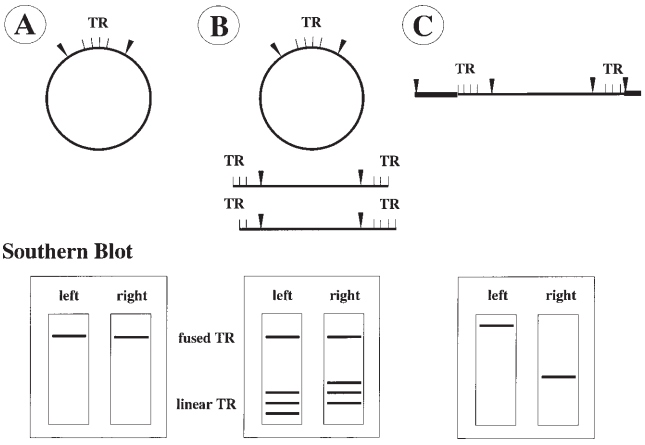

Fig. 4A–C. Possible intracellular EBV genomic structure and the expected results of Southern blot analysis [36,37]. Southern blot analysis can be used to distinguish circular, linear, and integrated viral genomic structures by hybridizing *Bam* H I-digested DNA with probes from either end of the linear genome (as indicated in *Fig. 3B*). The terminal repeat sequences are indicated by *hatched lines* and the *Bam* H I cut sites are indicated by *arrowheads*. **A** Episomal EBV DNA, with an enzyme such as *Bam* H I that spares the TR sequences, produces a single fused terminal fragment in monoclonal cells. **B** Permissively infected cells have a ladder array of small terminal fragments representing linear viral DNA. **C** When the virus is integrated through the terminal repeat (*TR*), distinct restriction fragments representing viral/cellular junction fragments are detected

As for the expression of viral latent genes, immunoblotting and immunocytochemistry have revealed the expression of EBNA1 but not EBNA2 or LMP1 in EBVaGC [8,9,25,26], although some investigators reported LMP1 immunoreacivity in several patients with EBVaGC [28,29]. Reverse transcription (RT)-PCR analysis demonstrated that the expression pattern of EBVaGC was similar to that of a latency I neoplasm, such as Burkitt lymphoma [8,9,38]. The restricted expression of viral latent genes may confer nonsusceptibility to EBV-specific CTL recognition in tumor cells, since the function of EBV-specific CTL has been shown to be retained in patients with EBVaGC [9].

There are two subtypes of EBV — A and B — which differ not only in the sequences of the EBNA2 region but also in their capacity to immortalize B lymphocytes. Restriction fragment length polymorphism (RFLP) analysis also distinguishes between types C and D in the *Bam* HI I region, and between F and an f variant in the *Bam* HI F region (Fig.3). The f variant is found in NPC tissues, while type F is predominant in normal mucosa in Southern China [39]. The predominant EBV subtype

in EBVaGC among Japanese is type A with a type CF variant [8,40], which is also the predominant type in the throat washings of healthy controls in Japan [40]. Therefore, the dominant EBV strain in EBVaGC may reflect the dominant type prevalent in the general population under investigation. Recently, a deletion mutant in the LMP1 gene of EBV-DNA has been reported to occur predominantly in NPC [41]. However, in Japan, this type of EBV is predominant in the general population [42,43], and is commonly identified in EBVaGC [43], as well as in EBV-associated lymphomas (Y. Hayashi et al., manuscript in preparation).

Unanswered questions and controversies regarding EBVaGC

EBV Infection in stomach epithelial cells

EBV infects B lymphocytes through the complement receptor, CD21. However, it has not yet been clarified how EBV infects epithelial cells which lack the CD21 molecule. Two possible mechanisms have been proposed. (1) There may be a receptor molecule specific to epithelial cells, as suggested by Yoshiyama et al. [44]; EBV with the neomycin-resistant gene efficiently infected cell lines of gastric carcinoma under selective pressure, and this could not be blocked by anti-CD21 monoclonal antibody. Cell-to-cell contact dramatically facilitated this process [45]. (2) Alternatively, there may be a mechanism involving immunoglobulin A (IgA)-mediated internalization as postulated by Sixbey and Yao [46]; EBV-specific IgA binds to viral particles in the mucosa and IgA-EBV complex is engulfed in confined epithelial cells, in which cell polarity is altered, based on prior cytopathology [47].

It has not yet been determined either how often or what type of epithelial cells are primarily infected with EBV in the stomach. In our study using EBER-ISH [8], we found that shedding epithelial cells of the fundic gland mucosa were positive in solitary or cluster form only in patients with a high titer of anti-EBV antibodies. In cultured lymphocytes, EBER1 is expressed 70 h after infection by EBV, and a similar finding has been reported in the EBV infection of CD21 (EBV-receptor)-transfected epithelial cells [48]. A cell kinetic study in gastric mucosa, however, showed that the foveolar epithelium moved upward after replicating in the neck region. It takes 36–192 h for epithelial cells to reach the tip of the foveola and to shed into the stomach lumen [49]. Thus, we speculate that EBV may infect some proliferating cells or surface epithelium-committed cells, possibly through EBV-carrying lymphocytes, and that the infected cells are shed when EBER is expressed in the infected cells.

In contrast to the above hypothesis, several researchers have recently raised the possibility that EBV infection of the stomach mucosa is not rare. Using DNA-ISH instead of EBER-ISH, they demonstrated occasional positive signals in epithelial cells of intestinal metaplasia [50–52], which also showed immunoreactivity for LMP1 but negative signals for EBER [50]. However, these results are controversial; in addition to the technical problems which may be inherent with gastrointestinal tissues [53,54], we could not confirm their findings with DNA-ISH using a probe from the same source and the same protocol. In latent infected cells, such as EBVaGC, the copy number of EBV per infected cells is generally considered to be 100, at most, which is the lower limit of defection with ISH with DNA probes without any enhancing procedure. The infection in the epithelial cells of intestinal metaplasia could have been replicative, because the signals presented in the studies above [50,52] were too strong if EBV had been in the latent phase. However, in the application of PCR for the *Bam* HI W region of EBV-DNA to microdissected tissues of non-neoplastic gastric mucosa, 2 of 118 microdissected samples from stomachs with EBVaGC and 5 of 62 samples from those with EBV-negative gastric carcinoma showed amplification of EBV-DNA, 3 being pyloric and 4, fundic, while none of the metaplastic gland samples showed such amplification (Kaizaki et al., manuscript submitted). This finding suggests that replicative infection of intestinal metaplasia is unlikely. Thus, at present, we believe that EBV infection is a rare event in the stomach, and that the primary target of EBV infection is not the epithelial cells of metaplastic glands.

Carcinogenesis and morphogenesis of EBVaGC

As for the predisposing factors for EBVaGC, both systemic and local factors should be taken into consideration. Significantly high titers of serum IgA antibodies to viral capsid antigen (VCA) have been reported to be a serologic feature in patients with EBVaGC [8,9]. Levine et al. [55] observed a high titer of serum anti-IgG EBV-VCA antibody in patients with EBVaGC more than 10 years before surgery. However, there has been no direct evidence of altered T-cell immunity against EBV. Imai et al. [9] demonstrated that EBV-specific CTL activity was similar in patients with EBVaGC, patients with EBV-negative carcinomas, and age-matched healthy blood donors. Although a protective association was observed between NPC and a major histocompatibility complex (MHC) class I antigen (human leakocyte antiger [HLA]-A2) in United States Caucasians [56], the HLA-A2 type was observed in approximately 60% of Chinese and Japanese patients with EBVaGC, higher than the incidences reported in the

corresponding local populations [57]. The MHC class II antigen, HLA-DQ3, is considerably frequent in patients with EBVaGC [58], although its significance needs to be clarified in the context of immunity against viral infection.

As for a local predisposing factor, non-neoplastic mucosa of the proximal stomach bearing EBVaGC may have been conditioned to develop EBVaGC [19]. In this context, we histologically evaluated gastritis in non-neoplastic gastric mucosa which surrounded early carcinoma of EBVaGCs ($n = 23$) and EBV-negative carcinomas (intestinal type, $n = 139$; diffuse type, $n = 44$) (Kaizaki et al., manuscript submitted). Marked atrophy and moderate-to-marked lymphocytic infiltration were observed in 74% and 78% of EBVaGCs, 49% and 12% of intestinal-type EBV-negative carcinomas, and 27% and 12% of diffuse-type EBV-negative carcinomas, respectively ($P < 0.05$). Only 13% of EBVaGCs were surrounded by intestinal metaplasia, in contrast to 41% of intestinal-type EBV-negative gastric carcinomas. EBVaGC may develop from EBV-infected epithelial cells in severe atrophic gastritis, but this process is not directly related to intestinal metaplasia. Based on the theory of an IgA-mediated internalization mechanism, it is reasonable to assume that the primary target of EBV infection is not polarized epithelial cells of intestinal metaplasia, but, rather, stem cells of the fundic gland in severe gastritis, which may lose their polarity for differentiation.

There are three possible mechanisms (Fig. 2) by which EBV may be related to cancer initiation in the stomach [8]. (A) EBV may be the sole factor that initiates EBVaGC. If mucosal damage delays the flow of epithelial cells, then EBV-infected cells can grow within the gland. (B) EBV may cooperate with other promoting factors. EBV-infected cells may be prone to subsequent genetic alterations, which initiate carcinomatous growth. (C) Alternatively, proliferating cells, which have already started neoplastic growth but remain a small fraction within the mucosa, may be more likely to be infected by EBV. This mechanism is based on the assumption that EBV-infected cells have some advantage for monoclonal growth over other, uninfected, cancer cells. The expression of latency genes in EBVaGC is latency type I, suggesting that EBV does not play a positive role in the maintenance of carcinoma cells. This assumption is challenged by the finding that a Burkitt lymphoma cell line, Akata, loses its tumor-forming capacity when it loses EBV from its nucleus [59]. However, it has not yet been clarified whether this phenomenon is specific to Akata, and which viral protein is responsible for the malignant phenotype of Akata.

Studies of the molecular mechanism underlying EBVaGC have only recently begun, compared with those of NPC [60,61]. Using PCR-RFLP and microsatellite markers, we observed that deletion of 5q and/or 17p and microsatellite instability were extremely rare in EBVaGC, in contrast to their high frequency in EBV-negative carcinoma, particularly its intestinal type [62]. This indicates that the genetic pathways of EBVaGC and EBV-negative carcinoma may be different. An immunohistochemical study reported that EBVaGC was independent of *bcl*-2 expression and p53 accumulation [63].

Few studies have investigated the cellular characteristics of EBVaGC in detail. Both the frequency of apoptosis and the proportion of proliferative cells were significantly lower in EBV-associated lymphoepithelioma-like carcinoma than in conventional EBV-negative gastric carcinomas [64]. However, whether the determinant of these phenomena was the presence of EBV or the particular histologic type of gastric carcinoma was not clear. Mucin histochemistry has revealed gastric type mucin to be predominant in most EBVaGC [58]. Some isoforms of CD44, an adhesion molecule of the cell surface, have been associated with the metastatic potential of carcinomas, such as colon and breast carcinomas. When CD44 variants 3–5 and 6 were immunohistochemically determined in gastric carcinoma, a multivariate analysis showed that EBV association and lymph node metastasis contributed independently to CD44 variant-expression [65]. Thus, the mechanism and significance of CD44 variant expression are different in gastric carcinoma with and without EBV. It is possible that EBV infection may influence CD44 expression by interacting with cytokine genes, such as those for tumor necrosis factor (TNF)α, interferon (INF)γ and interleukin 10, which are known to modulate CD44 expression. Infiltration of lymphocytes, most of which are CD8-positive in EBVaGC [58], may be induced by such a mechanism [66], rather than as a reaction to carcinoma cells.

Model systems for EBVaGC

Several experimental systems are now being employed for the study of EBVaGC, such as an in-vitro infection system, using the virus-producing cell line Akata, and genetically engineered EBV [44,45] to investigate the mechanism of EBV infection in epithelial cells. In-vitro cell culture and in-vivo transplantation of neoplastic cells which retain the characteristics of the original tumor are also useful for studying the cell biology and molecular mechanism underlying EBVaGC. Since a stable cell line of NPC that carries the EBV genome in its nuclei has not yet been established, we attempted to transplant a human EBVaGC in severe combined immunodeficiency (SCID) mice. We established a car-

cinoma, designated KT after the patient from whom the tumor was derived [67]. Mucin- and cytokeratin-expression and *Alu* sequence in tumor DNA confirmed that the KT tumor was derived from human epithelial tissue. The identity of clonal EBV in the original and KT tumors was demonstrated by TR analysis of EBV-DNA. The pattern of latency gene expression of EBV was the same in both tumors: EBER1 was also found in tumor cell nuclei by ISH. Reverse transcription-PCR analysis also demonstrated Qp-driven EBNA1 expression, but not EBNA2- or LMP1-expression. Thus, the transplantable human EBVaGC retains the original EBV with the same latency gene expression, and serves as a model system. Future experimental systems could include a transgenic mouse model [68], which expresses a viral protein, such as EBNA1, in gastric mucosa. Examining the susceptibility to chemical carcinogens in a transgenic mouse model should help to further clarify the genetic changes and reveal the molecular events underlying EBVaGC.

As for a therapeutic approach, Gutierrez et al. [69] investigated the possibility of gene therapy for EBV-associated neoplasm. Upon transfer of *Zta* (gene coding Zta/BZLF1/ZEBRA) into EBV-positive lymphoma cell lines, the latent virus switched to a lytic cycle, which resulted in lysis of the neoplastic cells.

Concluding remarks

EBVaGC is a unique type of gastric carcinoma which is tagged by clonal EBV, and may become a relatively more important gastric carcinoma entity as other risk factors decline. Future studies should examine the molecular events in the development of EBVaGC, including the role of EBV, the interaction of EBV with cytokine genes, the identification of predisposing factors, and the establishment of a therapeutic strategy. Gene therapy specific to EBV-associated neoplasm, if established, should establish EBVaGC as a distinct clinical entity in gastric cancer.

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