Spatial specificity of Epstein-Barr virus infection in gastric cancer: A 44-case clinicopathological analysis

XUMING WANG[1,2], LONGKUAN XU[1], XIAOZHEN YANG[1], XIAOLU YUAN[3,4], HOULIANG LU[5], QIONG LIU[6] and LIJIANG LIU[6]

[1]Department of Pathology, The First Affiliated Hospital of Guilin Medical University, Guilin, Guangxi 541001, P.R. China; [2]Department of Pathology, Wuhan Fourth Hospital (Wusheng Road Campus), Wuhan, Hubei 430014, P.R. China; [3]Department of Pathology, The First Affiliated Hospital of Chengdu Medical College, Chengdu, Sichuan 610500, P.R. China; [4]Department of Pathology, Maoming People's Hospital, Maoming, Guangdong 525000, P.R. China; [5]Department of Pathology, Laibin People's Hospital, Laibin, Guangxi 546100, P.R. China; [6]Department of Pathology, Wuhan Sixth Hospital, Wuhan, Hubei 430014, P.R. China

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Abstract. Epstein-Barr virus (EBV) is detected in ~10% of gastric cancer (GC) cases, but whether it directly drives carcinogenesis or merely colonizes established tumors remains unclear. The present study aimed to characterize the clinicopathological features and infection patterns of EBV-positive GC (EBVaGC) and to explore their translational implications. EBV-encoded RNA *in situ* hybridization was performed to identify EBVaGC cases among 456 patients with GC. Immunohistochemistry was used to assess Ki-67, proliferating cell nuclear antigen (PCNA) and claudin 18.2 (CLDN18.2) expression. Clinicopathological characteristics and survival data were analyzed. Of the 456 patients, 44 (9.6%) were EBV-positive. EBVaGC predominantly occurred in men and often presented as focal ulcerative masses with diverse histologies. No significant differences were observed in terms of age, tumor size, tumor/node stage, or long-term prognosis between EBV-positive and -negative groups (P>0.05), although EBV-positive patients showed better short-term outcomes (defined as 12-month survival rate; P=0.046). Notably, EBV infection was spatially restricted, being detected in cancer cells and in a subset of adjacent high-grade intraepithelial neoplasia (HGIN) tissues (25%), but absent in all non-neoplastic gastric mucosal cells examined, including chronic gastritis, low-grade intraepithelial neoplasia without concurrent GC and high-grade intraepithelial neoplasia (HGIN) without concurrent GC. Ki-67, PCNA and CLDN18.2 expression did not differ by EBV status. In conclusion, EBV infection in GC exhibits a unique spatial pattern, present in neoplastic but not normal epithelium, suggesting it may be a late event rather than a direct etiological driver. This infection pattern provides a rationale for EBV-directed targeted therapies, as the virus could serve as a tumor-specific molecular marker.

Correspondence to: Professor Xuming Wang, Department of Pathology, Wuhan Fourth Hospital (Wusheng Road Campus, 473 Hanzheng Street, Qiaokou, Wuhan, Hubei 430030, P.R. China
E-mail: 107967594@qq.com

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Introduction

Gastric cancer (GC) remains one of the most common malignancies worldwide, with >968,000 new cases and nearly 660,000 associated deaths in 2022 (1). Epstein-Barr virus (EBV) is detected in ~10% of GC cases and defines a distinct molecular subtype (2,3). However, the exact role of EBV in gastric carcinogenesis remains debated. Two competing hypotheses exist: EBV may act as a direct oncogenic driver in normal epithelial cells, or it may secondarily infect cells that have already undergone neoplastic transformation (4,5).

Distinguishing between these possibilities has important clinical implications. If EBV is a direct driver, antiviral or EBV-targeted therapies could be deployed for prevention or early intervention. Conversely, if EBV infection occurs after malignant transformation, it may serve as a tumor-specific marker for targeted therapies, given that EBV-positive cells are confined to neoplastic tissue.

Previous studies have described the clinicopathological features of EBV-positive GC (EBVaGC), such as male predominance, proximal location and lymphoepithelioma-like histology (6-8). However, most have focused on descriptive epidemiology rather than the spatial relationship between EBV infection and normal vs. neoplastic epithelium. Whether EBV selectively infects cancer cells or also colonizes adjacent normal mucosa remains poorly characterized.

In the present study, 456 GC cases were analyzed to identify EBVaGC by Epstein-Barr encoding region-*in situ* hybridization (EBER-ISH). The study aimed to i) characterize the clinicopathological features of EBVaGC, ii) compare survival outcomes between EBV-positive and -negative patients, and

iii) determine the spatial pattern of EBV infection within tumor, adjacent precancerous lesions and normal mucosa. The findings challenge the direct causation hypothesis and highlight the translational potential of EBV as a tumor-specific target for precision therapy.

Patients and methods

Patients. A total of 456 paraffin-embedded GC specimens were collected from patients who underwent surgical resection between January 2010 and December 2024, and were subsequently retrieved from the pathology archives for the present study. These samples consisted of 376 cases from the Department of Pathology at the First Affiliated Hospital of Guilin Medical University (Guilin, China) and 80 cases from the Department of Pathology at Maoming People's Hospital (Maoming, China). Additionally, to investigate the spatial distribution of EBV infection across the gastric carcinogenesis spectrum, 56 samples of chronic superficial gastritis, 70 of chronic atrophic gastritis, 50 of low-grade intraepithelial neoplasia (LGIN) without concurrent GC and 28 of high-grade intraepithelial neoplasia (HGIN) without concurrent GC were collected from the Department of Pathology at the First Affiliated Hospital of Guilin Medical University during the same period. As positive controls for EBER detection, 10 paraffin-embedded samples of non-keratinizing undifferentiated squamous cell carcinoma of the nasopharynx and 8 samples of Burkitt lymphoma were collected. All specimens were fixed in 10% neutral buffered formalin at room temperature for 6-24 h, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin (H&E) at room temperature (hematoxylin, 5 min; eosin, 1 min). Morphological evaluation was performed under an Olympus BX53 light microscope (Olympus Corporation). Ethical approval was obtained from the Ethics Committees of the First Affiliated Hospital of Guilin Medical University (approval no. 2025IITLL-61) and Maoming People's Hospital (approval no. PJ2020MI-K183-01). Written informed consent was provided by all participants. HGIN was diagnosed according to World Health Organization 2019 criteria (9). Malignant transformation was distinguished from HGIN by the presence of marked nuclear atypia, loss of polarity and cribriform architecture, which are consistent with the Japanese Classification for Intramucosal Carcinoma (early GC) (10). The inclusion criteria for GC specimens were as follows: No preoperative radiotherapy or chemotherapy, absence of distant metastasis (M0), and complete documentation of age, sex, maximum tumor diameter, T stage, N stage, postoperative treatment, gross type and histological type. The exclusion criteria included incomplete clinical history, pathological information, treatment records or follow-up data. All pathological diagnoses were reviewed by two senior pathologists.

Main reagents. The EBER ISH kit (cat. no. EBV-0100), Ki-67 mouse monoclonal antibody (cat. no. MAB-0672), proliferating cell nuclear antigen (PCNA) mouse monoclonal antibody (cat. no. MAB-0145), claudin 18.2 (CLDN18.2) rabbit monoclonal antibody (cat. no. RAM-1088), ready-to-use MAXVision™ immunohistochemical detection kit (cat. no. KIT-5030), IHC Biotin Block Kit (cat. no. BLK-0002), antigen retrieval solution (cat. no. MVS-0099), enzyme substrate chromogen (cat. no. DAB-2031) and digoxin staining solution (cat. no. MC-3002) were purchased from Fuzhou Maxin Biotechnology Co., Ltd. The Ki-67 mouse monoclonal antibody was used at a dilution of 1:200 in antibody diluent (cat. no. ABD-0030; Fuzhou Maxin Biotechnology Co., Ltd.). The PCNA mouse monoclonal antibody, CLDN18.2 rabbit monoclonal antibody and the EBER ISH kit were ready-to-use and applied according to the manufacturer's instructions.

Immunohistochemical experimental procedure and interpretation. After deparaffinization of paraffin-embedded sections in xylene and rehydration through a graded ethanol series, antigen retrieval was performed in boiling EDTA solution using a pressure cooker at 121˚C for 3 min. Endogenous peroxidase activity was blocked with 5% BSA (cat. no. A8020; Beijing Solarbio Science & Technology Co., Ltd.) at room temperature for 30 min. Sections were then incubated with primary antibodies for 60 min at room temperature, followed by incubation with the MAXVision detection reagent for 15 min. DAB was used for visualization. Counterstaining was performed with hematoxylin at room temperature for 5 min. For routine morphological evaluation, H&E staining was performed separately at room temperature: Hematoxylin staining was performed for 5 min, followed by eosin staining for 1 min. Morphological evaluation was performed under an Olympus BX53 light microscope (Olympus Corporation). Ki-67 and PCNA were assessed by nuclear staining, and CLDN18.2 by membrane/cytoplasmic staining. Staining intensity was scored as follows: 0, no staining; 1, light yellow; 2, brown-yellow. The percentage of positive cells out of 500 tumor cells was multiplied by the intensity score to generate a final score (0-200%). Cell positivity was defined as the presence of clearly distinguishable nuclear (for Ki-67 and PCNA) or membranous/cytoplasmic (for CLDN18.2) staining.

ISH experimental procedure and interpretation. EBER ISH was performed on 3-μm paraffin-embedded sections using a digoxin-labeled probe (commercially purchased probe, included in the aforementioned EBER ISH kit) according to the manufacturer's instructions. The probe sequence is proprietary to the manufacturer and not publicly available. After deparaffinization and protease digestion, the probe was applied and sections incubated overnight at 37˚C. DAB was used for chromogenic detection, followed by hematoxylin counterstaining at room temperature for 5 min. Brown-yellow nuclear staining was considered positive. Positive controls were nasopharyngeal carcinoma and Burkitt lymphoma tissues.

Pathological morphology assessment and digital slide preparation. All H&E-stained, immunohistochemically stained and ISH-stained slides were scanned using a Jiangfeng digital slide scanner (KF-PRO-005; Ningbo Jiangfeng Bioinformatics Technology Co., Ltd.) to generate high-resolution SVS format images. Morphological evaluation was performed under an Olympus BX51 microscope (Olympus Corporation) by two senior pathologists, who independently reviewed all slides in a double-blind manner. When disagreements arose, consensus

was reached through discussion; if no consensus could be reached, a third senior pathologist with 20 years of experience made the final decision.

Survival analysis and protein expression matching. To minimize confounding factors, EBV-negative cases were propensity-matched to EBV-positive cases based on sex, histological type/grade, TNM stage and treatment modality. Histological type and grade were classified according to the World Health Organization Classification of Digestive System Tumours (5th edition, 2019) (9). TNM stage was determined according to the Union for International Cancer Control/American Joint Committee on Cancer TNM staging system (8th edition) (11). Age and maximum tumor diameter were also matched as closely as possible. Two-sample t-tests confirmed no significant differences in age or tumor size between the matched groups. Paired t-tests were used for comparing continuous variables (age and tumor diameter) between matched pairs in Table SI. Survival analysis used log-rank, Breslow and Tarone-Ware tests. Comparisons of Ki-67, PCNA and CLDN18.2 expression between matched groups were performed using unpaired t-tests, as the matched pairs from the survival analysis did not all have available tissue for IHC, resulting in different sample sizes for each marker. Pearson's χ^2 or Fisher's exact tests were used for categorical variables, including T stage and N stage when analyzed as 4x2 contingency tables). McNemar's test was used for paired 2x2 categorical variables (sex and postoperative chemotherapy variables in Table SI). Two-tailed $P<0.05$ was considered to indicate a statistically significant difference. Based on the 10% expected incidence of EBVaGC (12), the 44 positive cases are within the expected range for a single-institution study; formal power analysis was not performed.

Results

Spatial specificity of EBV infection in gastric carcinogenesis. EBV infection was strictly confined to neoplastic epithelium. ISH revealed that EBER-positive signals (brown-yellow nuclear granules) were present in 44 out of 456 (9.6%) GC samples. Notably, within EBVaGC tissues, infection was spatially restricted: EBV-positive cancer cells formed distinct, continuous patches with clear boundaries from EBV-negative areas, with no intermixing (Fig. 1).

Crucially, EBV was absent in all non-neoplastic gastric mucosal cells examined, including 56 cases of chronic superficial gastritis, 70 cases of chronic atrophic gastritis with intestinal metaplasia, 50 cases of LGIN and 28 cases of HGIN without concurrent GC (Table I).

By contrast, among the 44 EBVaGC cases, EBV expression was detected in adjacent HGIN (intramucosal carcinoma) tissues in 11 cases (25%). This finding suggests that in these cases, the areas initially diagnosed as HGIN may had already undergone malignant transformation and thus became susceptible to EBV infection. EBV was also sporadically detected in infiltrating lymphocytes in a subset of non-cancer samples (e.g., 13/56 chronic gastritis cases), but never in gastric epithelial cells outside of cancer or HGIN with malignant transformation.

Clinicopathological features of EBVaGC. EBVaGC was more prevalent than EBV-negative cases in male patients (male:female ratio, 7.8:1 vs. 2.25:1) ($P=0.007$). In the EBV-positive group, the mean age was 62.23±9.83 years (range: 46-85 years). No significant differences were observed between EBV-positive and -negative groups in terms of age, maximum tumor diameter, T stage or N stage (all $P>0.05$) (Tables II-IV). Grossly, EBV-positive tumors often presented as focal ulcerative masses, with only one case showing a protruding morphology. Histologically, tubular adenocarcinoma (24/44) and poorly differentiated solid-type adenocarcinoma (14/44) were the most common subtypes. Representative images of the gross morphology and poorly differentiated solid-type adenocarcinoma are shown in Fig. S1A and B, respectively.

EBV status does not alter proliferation or CLDN18.2 expression. To assess whether EBV infection impacts tumor cell biology, the expression of Ki-67, PCNA and CLDN18.2 was compared in EBV-positive vs. matched EBV-negative GC cases. After rigorous matching for potential confounders (age, sex, tumor size, TNM stage and histology), unpaired t-tests revealed no significant differences in any of the three markers between the two groups (all $P>0.05$) (Table V; Fig. S2). As tissue availability was limited, the sample sizes for IHC analysis varied: 30 pairs for Ki-67, 37 for PCNA and 38 for CLDN18.2. The results suggest that EBV status does not substantially alter these basic cellular or potential therapeutic (CLDN18.2) characteristics.

Short-term survival benefit but no long-term prognostic impact. Survival analysis of 24 matched patient pairs showed that EBV-positive patients had significantly higher short-term survival rates (defined as the first 12 months after surgery) compared with EBV-negative patients (Breslow test; $P=0.046$). The baseline characteristics of the 24 matched pairs are shown in Table SI. Only 24 pairs could be exactly matched on sex, histology, TNM stage and treatment; the remaining 20 EBV-positive cases lacked suitable matches and were therefore excluded from the matched survival analysis. However, this survival advantage was not sustained in the medium-to-long term (beyond 12 months), as assessed by the log-rank and Tarone-Ware tests ($P=0.087$ and $P=0.059$, respectively) (Table VI; Fig. S1C). This suggests EBV positivity may confer an early benefit but does not alter overall long-term prognosis.

Discussion

The present study reports a previously underappreciated but potentially important observation regarding EBV infection in GC, namely, spatial specificity. By systematically examining a large cohort of GC samples alongside a spectrum of precancerous lesions, it was found that EBV infection is strictly confined to neoplastic epithelium. EBV infection was consistently present in cancer cells and, in 25% of cases, in adjacent HGIN tissues (which may already harbor malignant transformation), but was completely absent from normal gastric mucosa, chronic gastritis, LGIN and HGIN without concurrent GC. This unique infection pattern challenges the prevailing hypothesis that EBV acts as a direct etiological driver and instead points to a new paradigm: EBV selectively

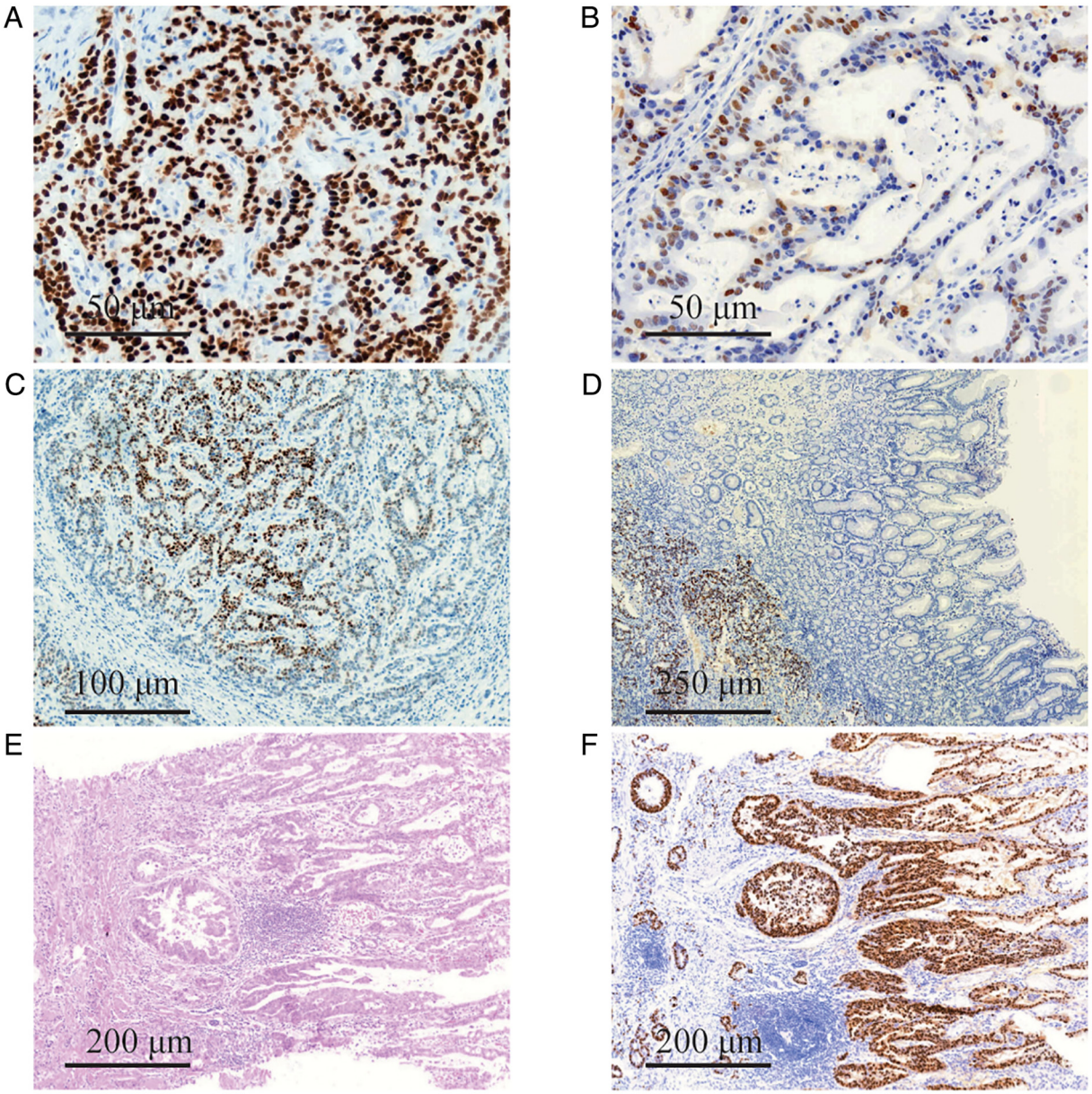

Figure 1. EBER *in situ* hybridization and corresponding H&E staining. (A) EBER-positive staining localized in the nuclei of GC cells (magnification, x200). (B) Approximately 50% of GC cells exhibit EBER expression (magnification, x200). (C) Variable intensity of EBER expression within glands; some cells are negative (magnification, x100). (D) Normal gastric mucosa adjacent to the tumor shows no EBER staining (magnification, x40). (E) H&E staining of a lesion initially suspected to be high-grade intraepithelial neoplasia, re-evaluated as intramucosal carcinoma (well-differentiated tubular adenocarcinoma) based on marked nuclear atypia, loss of polarity and cribriform architecture (magnification, x50). (F) The same area shows strong EBER positivity (brown nuclear signal) (magnification, x50). GC, gastric cancer; EBER, Epstein-Barr encoding region; H&E, hematoxylin and eosin.

colonizes cells that have already undergone malignant transformation. This finding has profound biological and translational implications.

The spatial specificity of EBV infection provides the strongest evidence to date against the direct causation hypothesis. If EBV were an initiator of gastric carcinogenesis, it should be detectable in normal or premalignant gastric epithelium. The present systematic analysis across 456 GC cases and 204 non-cancerous/precancerous specimens revealed no EBV in normal epithelial cells, LGIN or HGIN without concurrent GC. This is consistent with the observation that EBV genomes exist as episomes rather than integrating into the host chromosome in GC (13,14), and with reports that EBV fails to infect normal gastric epithelial cells *in vitro* (15). We therefore propose that, rather than being an initiator, EBV infection in GC is a secondary event that occurs after malignant transformation has been established. The detection of EBV in a subset of adjacent HGIN tissues (25%), which are likely to have already undergone malignant transformation, suggests that this colonization occurs at the HGIN stage. This is consistent with Japanese diagnostic criteria, which classify such lesions as early cancer (16), and further supports the notion that EBV selectively targets cells that have already acquired neoplastic properties.

Although EBV-positive lymphocytes were observed in some non-cancer samples (e.g., 13/56 chronic gastritis cases), there was no spatial correlation between lymphoid EBV positivity and adjacent epithelial infection. Moreover, EBV

Table I. Positive rate of Epstein-Barr virus infection in normal gastric mucosa, chronic gastritis, low-grade and high-grade dysplasia of gastric mucosa, and invasive GC.

Types	Total number of cases	Number of positive cases	Positive rate, %
Chronic superficial gastritis	56	0	0
Chronic atrophic gastritis	70	0	0
LGIN	50	0	0
HGIN	28	0	0
Invasive GC	456	44	9.6

LGIN, low-grade intraepithelial neoplasia; HGIN, high-grade intraepithelial neoplasia; GC, gastric cancer.

Table II. Comparison of clinical and pathological features between EBV-positive (n=44) and -negative (n=412) gastric cancer.

Group	EBV-positive, n	EBV-negative, n	P-value
Sex			0.007
Male	39	286	
Female	5	126	
T stage			0.257
Tis + T1	5	44	
T2	6	57	
T3	15	177	
T4	18	134	
N stage			0.658
N0	12	113	
N1	10	74	
N2	5	75	
N3	17	150	

EBV, Epstein-Barr virus.

Table III. Comparison of maximum tumor diameter between EBV-positive and -negative gastric cancer.

Parameter	EBV-positive (n=44)	EBV-negative (n=405)[a]	P-value
Maximum tumor diameter, cm	5.13±2.27	4.98±2.67	0.716

[a]Tumor diameter was not recorded in 7 EBV-negative cases; they were excluded from this analysis. EBV, Epstein-Barr virus.

Table IV. Comparison of age at diagnosis between patients with EBV-positive and -negative gastric cancer.

Parameter	EBV-positive (n=44)	EBV-negative (n=412)	P-value
Age, years	62.23±9.83	61.31±10.15	0.567

EBV, Epstein-Barr virus.

was never detected in normal gastric mucosa. Therefore, lymphocyte-to-epithelium seeding is unlikely to be a major mechanism of EBV infection in GC. The detection of EBV in adjacent HGIN (25%) suggests that these lesions may have already acquired neoplastic properties, consistent with Japanese diagnostic criteria. However, this inference is based on spatial association, and independent molecular evidence (e.g., clonality analysis) would be required to confirm transformation.

The spatial specificity of EBV infection has a direct and compelling translational implication: EBV represents a near-ideal tumor-specific molecular marker. EBV-specific T-cell therapy is already in clinical trials for nasopharyngeal carcinoma, and the present findings support extending this approach to EBVaGC. In an era of precision oncology, the greatest challenge is often the lack of targets that are uniformly expressed in cancer cells but absent in normal tissues. The finding that EBV is consistently present in EBVaGC cells but absent in all adjacent normal gastric mucosa suggests that EBV itself, or the pathways it engages, could be exploited for highly specific therapeutic interventions.

This is fundamentally different from most current targeted agents, which often target overexpressed but not truly cancer-specific proteins, leading to on-target, off-tumor toxicities. Harnessing EBV as a therapeutic tool could take multiple forms: i) EBV-specific cytotoxic T lymphocytes, already explored in EBV-associated lymphomas and nasopharyngeal carcinoma (17), could be redirected to EBVaGC; ii) the natural tropism of EBV for neoplastic cells could be exploited as a delivery vehicle for suicide genes or oncolytic viruses; and iii) the viral antigens themselves could serve as targets for chimeric antigen receptor-T cells. The observation that EBV infects GC cells in contiguous patches, with clear boundaries from uninfected areas, suggests a mechanism of cell-to-cell spread that may facilitate complete tumor coverage by such EBV-targeted therapies.

Together, these findings suggest that EBV positivity serves as a reliable indicator of neoplastic transformation: In gastric mucosa, the presence of EBV in epithelial cells marks cells that have already undergone malignant change, even when morphological features are equivocal.

The present study also helps clarify previously conflicting reports on the prognostic impact of EBV status (18-20). A large-scale meta-analysis by Pyo *et al* (18) demonstrated that patients with EBVaGC had better overall survival (higher survival rates) compared with patients with EBV-negative GC (HR, 0.890; 95% CI, 0.816-0.970). Another meta-analysis

Table V. Immunohistochemical analysis of Ki-67, PCNA and CLDN18.2 expression in EBV-positive and -negative gastric cancer (matched pairs).

Marker	EBV-positive patients, n	EBV-negative patients, n	Mean positive cells ± SD (positive group), %	Mean positive cells ± SD (negative group), %	t value	P-value
Ki-67 expression	30	30	0.58±0.23	0.55±0.23	0.62	0.537
PCNA expression	37	37	1.23±0.63	1.16±0.54	0.51	0.607
CLDN18.2 expression	38	38	18.7±7.4	15.8±6.9	0.28	0.777

IHC analysis was performed on available tissue blocks. Sample sizes vary due to limited tissue cores for certain markers. All values represent the percentage of positive cells (mean ± SD). All comparisons used unpaired t-tests.

Table VI. Survival analysis for Epstein-Barr virus-positive and -negative groups.

Test	χ^2	P-value
Log-rank	2.935	0.087
Breslow (generalized Wilcoxon)	3.972	0.046
Tarone-Ware	3.564	0.059

focusing on GC with lymphoid stroma similarly reported lower mortality rates in EBV-positive cases (19). However, subgroup analyses from the meta-analysis performed by Pyo *et al* (18) revealed that the prognostic benefit of EBV positivity was not universal: it reached statistical significance in Asian and American cohorts, whereas the European cohort did not show a significant benefit (HR, 0.915; 95% CI, 0.814-1.028) (18). Furthermore, when compared within the context of molecular classification, EBV-positive tumors showed no significant survival difference compared with microsatellite instability or microsatellite stable subtypes (18). These discrepancies likely stem from inadequate control for confounding factors such as TNM stage, histological subtype and treatment modalities, limitations that the rigorous matching approach in the present study was designed to address. The finding of no long-term survival difference in the present Chinese cohort is consistent with the geographic heterogeneity reported by Pyo *et al* (18).

By rigorously matching EBV-positive and -negative cases for all major confounders (age, sex, tumor size, TNM stage, histology and treatment), it was found that EBV positivity was associated with a transient short-term survival benefit (P=0.046) but did not affect long-term prognosis. This suggests that while EBV infection may alter early postoperative outcomes (possibly through immune modulation), it does not fundamentally change the aggressive potential of the tumor. This nuanced finding reconciles prior contradictory reports, a number of which did not adequately control for these confounding factors.

Similarly, the absence of any difference in Ki-67, PCNA or CLDN18.2 expression between EBV-positive and -negative groups indicates that EBV infection does not fundamentally alter basic tumor cell biology, further supporting the 'accompanying phenomenon' model. The lack of difference in CLDN18.2 expression is also clinically relevant, as it suggests that CLDN18.2-targeted therapies (such as zolbetuximab) may be equally applicable to patients with EBV-positive GC and EBV-negative GC.

The present study has several limitations. First, the relatively low incidence of EBVaGC (9.6%) resulted in a modest sample size (n=44), which may have limited statistical power for some subgroup analyses. Second, as a single-institution, retrospective study, the findings require validation in larger, prospective, multicenter cohorts with standardized EBV detection methods and extended follow-up. Third, while we propose a biological model (EBV as a secondary colonizer), the precise molecular mechanism by which EBV selectively infects malignant but not normal gastric epithelial cells remains unknown and warrants further investigation. Fourth, EBV latency type or viral integration status were not assessed, which could provide additional insight into the mechanism of selective infection. Unraveling these mechanisms could not only deepen our understanding of EBV biology but also open new avenues for preventing or exploiting this phenomenon therapeutically. Fifth, the lack of a formal power analysis due to the relatively low incidence (~10%) of EBVaGC may limit the statistical power of the subgroup analyses, and the modest sample size (n=44) should be interpreted with caution.

In conclusion, the present study reveals a previously underappreciated spatial specificity of EBV infection in GC. This finding challenges the direct causation hypothesis, supports a 'secondary colonization' model, and, most importantly, highlights EBV as a promising tumor-specific target for precision therapy. By shifting the perspective from 'EBV as a pathogen' to 'EBV as a tool', more effective and more safe novel treatment strategies may be unlocked for EBVaGC and potentially other EBV-associated malignancies.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XW, XYa and LL were responsible for conceptualization and supervision. XW and LL wrote the original draft, reviewed and edited the manuscript, and prepared the figures and tables. LX, XYa, XYu, HL and QL were responsible for methodology development, investigation (data collection and experiments) and validation (data verification). XW acquired funding. XWa and LL confirm the authenticity of all the raw data. All authors read and approved the manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from the Ethics Committees of the First Affiliated Hospital of Guilin Medical University (Guilin, China; approval no. 2025IITLL-61) and Maoming People's Hospital (Maoming, China; approval no. PJ2020MI-K183-01). All participants provided written informed consent for participation, and the study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient consent for publication

All patients provided written informed consent for publication.

Competing interests

The authors declare that they have no competing interests.

Use of artificial intelligence tools

During the preparation of this work, AI tools were used to improve the readability and language of the manuscript or to generate images, and subsequently, the authors revised and edited the content produced by the AI tools as necessary, taking full responsibility for the ultimate content of the present manuscript.

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