Patients with *Helicobacter pylori*-positive gastric cancer with human cytomegalovirus infection have a low tendency of advanced lymphatic metastasis in a Chinese population

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Abstract. Recognized as a group I carcinogen for gastric cancer (GC) and a factor involved in the development of GC, *Helicobacter pylori* serves a major part in GC research. However, most studies have focused on *H. pylori* itself, ignoring the complicated pathogenic microbiological environment of GC and neglecting the synergistic or antagonistic effects of *H. pylori* with other pathogenic microorganisms. Increasing evidence has revealed that the human cytomegalovirus (HCMV) is present in several types of tumors and serves an important role in the neoplastic process of certain human malignant tumors, including GC. The aim of the present study was to explore the role of HCMV and *H. pylori* co-infection in GC. HCMV and *H. pylori* infection was analyzed in paired gastric tumor and peri-tumoral tissues from 134 (98 male and 36 female) patients using PCR. The results revealed that a total of 74 (55.2%) patients had *H. pylori* infection, 58 patients (43.3%) had HCMV infection, and 34 (25.4%) patients had both HCMV and *H. pylori* infection. Univariate and multivariate analyses demonstrated that *H. pylori* infection was independently associated with advanced lymphatic metastasis [P=0.007; odds ratio (OR)=3.51]. Furthermore, compared with HCMV⁻/*H. pylori*⁻, neither HCMV⁺/*H. pylori*⁻ nor HCMV⁺/*H. pylori*⁺ were associated with metastasis, but HCMV⁻/*H. pylori*⁺ co-infection status was an independent risk factor for advanced lymphatic metastasis (P=0.005; OR=6.00). In conclusion, GC co-infected with HCMV and *H. pylori* exhibited a low tendency of lymph node metastasis. HCMV may interact with *H. pylori* to inhibit the process of lymphatic metastasis, and the mechanism requires further investigation.

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Introduction

Gastric cancer (GC) is one of the most common malignant neoplasms of the upper gastrointestinal tract in Asia, with an incidence rate of 64.6 per 100,000 individuals in 2012 and exhibiting substantial mortality (1). Gastric oncogenesis is a multistep process, involving non-atrophic gastritis, atrophic gastritis, intestinal metaplasia, dysplasia and eventually GC (2). The exact factors involved in the occurrence and development of GC are not yet fully understood, with the exception of *Helicobacter pylori* infection. *H. pylori* is a Gram-negative bacteria that is strongly associated with GC occurrence, and it has been recognized as a group I carcinogen by the International Agency for Research on Cancer in 1994 (3). Furthermore, the chronic inflammation resulting from *H. pylori* infection is believed to be a major step in the initiation and development of GC (4).

Despite the high incidence of *H. pylori* infection worldwide, only 1-3% of the *H. pylori*-infected individuals progress to GC (5), which suggests that other factors must be involved in GC etiology. Human cytomegalovirus (HCMV), a member of the herpesvirus family (6), is prevalent among the general population, with an infectious rate ranging between 50 and 100% (7). In the last two decades, HCMV has been reported to serve an important role in the neoplastic process of human malignant tumors, such as glioblastoma, salivary gland cancer, lung carcinoma, breast cancer, prostatic carcinoma, colorectal cancer and hepatocellular carcinoma (8-14). Our previous study revealed that HCMV was present in 50-69.61% of GC tissues (15). However, only a few studies have focused on the effect of HCMV on GC. Previous studies have revealed a difference in the expression levels of the UL133, UL135, UL136 and UL138 genes between normal gastric and tumor tissues (16,17). Additionally, an association between HCMV infection and lymphatic metastasis was observed in GC (15). Considering the high prevalence of *H. pylori* and its role in GC, the synergetic or antagonistic effects between HCMV and *H. pylori* could not be ignored when investigating the role of HCMV in GC. Therefore, the present study aimed to detect the infection with HCMV and *H. pylori* in patients with GC using PCR and to explore the potential association between them in GC.

Materials and methods

Patients, specimens and data collection. A total of 134 patients (98 males and 36 females; age range, 31-89 years; median age, 68 years) who underwent elective gastrectomy for GC at The Second Affiliated Hospital of Wenzhou Medical University (Wenzhou, China) between January 2017 and January 2018 were included in the present study. Paired gastric tumor and peri-tumoral tissues (≥10 cm from the negative reception margin) were collected. The histopathological diagnosis of gastric tumor and paired non-tumor specimens was confirmed following surgery by the pathological department of The Second Affiliated Hospital of Wenzhou Medical University. Once collected, specimens were deposited into tubes and preserved in liquid nitrogen immediately until further use. Patient clinicopatho-logical data, including sex, age, tumor size (the longest tumor diameter), tumor location, general type (ulcer or non-ulcer), tumor invasion, lymphatic metastasis, TNM stage, vessel invasion (defined by the pathological department using hematoxylin and eosin staining according to routine hospital protocols) and differentiation, were collected prospectively. Tumor invasion, lymphatic metastasis and TNM stage were defined according to the National Comprehensive Cancer Network Gastric Cancer Guidelines version 3.2015 (18). The present study was approved by the Human Research Ethics Committee of The Second Affiliated Hospital of Wenzhou Medical University, and written informed consent was provided by all participants.

Genomic DNA extraction. Total genomic DNA was extracted from 134 pairs of tumor and peri-tumoral tissue samples using a QIAamp DNA mini kit (Tiangen Biotech Co., Ltd.) according

to the manufacturer's protocol. DNA purity and concentration were quantified using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.). DNA samples were stored at -20°C until further use.

Detection of H. pylori and HCMV infection. *H. pylori* and HCMV infections were determined by PCR. The primers for the *H. pylori* genes 16S rRNA (19) and UreA (20), and for the HCMV genes UL47, UL56 and UL77 (15) are listed in Table I. GAPDH was used as an internal control. PCR was performed in a T100™ Thermal Cycler (Bio-Rad Laboratories, Inc.). The PCR mixture contained 1X Taq MasterMix (Tiangen Biotech Co., Ltd.), 1 μg DNA sample and 0.2 μM each specific forward and reverse primers in a final volume of 25 μl. The positive controls for *H. pylori* and HCMV detection were DNA from a GC tissue resected from a patient clinically diagnosed with *H. pylori* infection at The Second Affiliated Hospital of Wenzhou Medical University and a clinically isolated HCMV strain or AD169 (American Type Culture Collection), respectively. The negative control was sterile double-distilled water. After an initial denaturation step at 95°C for 5 min, the reaction mixtures were processed through 35 PCR cycles of dena-turation at 95°C for 30 sec, annealing for 30 sec at 58°C for UL47, UL56, UL77 and 16S rRNA or 50°C for UreA, and extension at 72°C for 1 min, followed by an additional extension step at 72°C for 10 min. The final PCR products were loaded on a 1.2% agarose gel for electrophoresis, using GelRed for visualization. If a band was detected at the right size (according to the relative position of the band and marker) and the sequencing results (performed by The Beijing Genomics Institute) indicated the correct sequence, the gene was considered to be present in the tissue. If one of the two *H. pylori* genes or one of the three HCMV genes was effectively amplified, the tissue was considered posi-tive for *H. pylori* or HCMV, respectively. Additionally, patients were considered to have *H. pylori* or HCMV infec-tion if *H. pylori* or HCMV was detected in their gastric tissues, regardless of its presence in the tumor or adjacent peri-tumoral tissue.

Statistical analysis. Statistical analysis was performed using SPSS version 22.0 (IBM Corp.). Univariate analyses were performed using the χ^2 test with Yates' correction or Fisher's exact test depending on the data type. Multivariate logistic regression analysis was performed to determine the independent risk factors. P<0.05 was considered to indicate a statistically significant difference.

Results

Detection of H. pylori and HCMV infection. The results obtained using different primers exhibited good consistency (Fig. 1A). As aforementioned, patients were considered to have *H. pylori* or HCMV infection if *H. pylori* or HCMV were detected in their gastric tissues, regardless of its pres-ence in the tumor or paired peri-tumoral tissue. Among the 134 patients, *H. pylori* was detected in 74 cases (55.2%), including 52 cases positive only in peri-tumoral tissues and 22 cases positive in both tumor and peri-tumoral

Table I. Primer information.

A, *Helicobacter pylori*

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Target size, bp
16S rRNA	CTTAACCATAGAACTGCATTTGAAACTAC	GGTCGCCTTCGCAATGAGTA	119
UreA	GCCAATGGTAAATTAGTT	CTCCTTAATTGTTTTTAC	411

B, Human cytomegalovirus

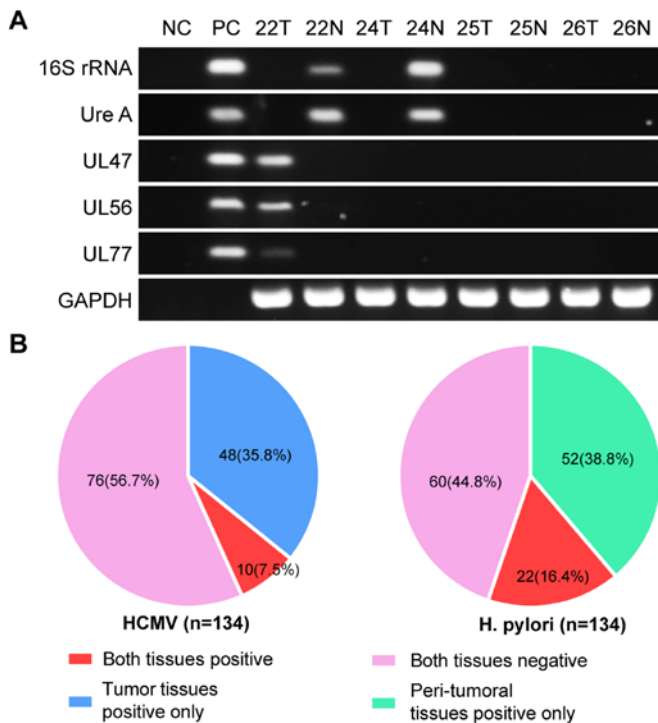| Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Target size, bp
UL47	GTACAGCCCCACGTTCCTG	CCCGATACAGGTACTCGCGCT	212
UL56	TCCTCCACGTCCTCCCCGTA	AGGCGCTGAGGGAGTACAAC	202
UL77	GCACTTTTGATCGTCACGTGCT	ACGCAGATATTGCTGTTCGTGC	215
GAPDH	CAGGGCTGCTTTTAACTCTGGTAA	GGGTGGAATCATATTGGAACATGT	101

bp, base pair.

Figure 1. Detection of HCMV and *H. pylori* in gastric tissues from patients with gastric cancer. (A) PCR products after agarose gel electrophoresis. Numbers above the lanes represent tumor or peri-tumoral specimens of patients. The NC is double-distilled water, and the PC is DNA from a clinically confirmed *H. pylori*+ gastric tissue and a clinically isolated HCMV strain (AD169). GAPDH was used as an internal control. (B) Infection rates of HCMV and *H. pylori* in 134 pairs of tissues from patients with gastric cancer. NC, negative control; PC, positive control; T, tumor specimen; N, peri-tumoral specimen; HCMV, human cytomegalovirus; *H. pylori*, *Helicobacter pylori*.

Table II. Association between HCMV and *H. pylori* in gastric tumors.

H. pylori status	Total	HCMV⁻	HCMV⁺	χ²	P-value
H. pylori⁻	60	36	24	0.477	0.490
H. pylori⁺	74	40	34		
Total	134	76	58		

HCMV, human cytomegalovirus; *H. pylori*, *Helicobacter pylori*.

10 cases positive in both tumor and peri-tumoral tissues (Fig. 1B). Both HCMV and *H. pylori* were detected in 34 cases (25.4%); however, there was no significant association between HCMV and *H. pylori* infection (P=0.490; Table II).

Associations between H. pylori infection and clinicopathological characteristics of patients with GC. Analysis of the clinicopathological characteristics revealed that patients in the *H. pylori*⁺ group exhibited a higher level of distant lymph node metastasis (N2 + N3) and TNM stage (T3 + T4), indicating that *H. pylori* infection status was significantly associated with lymphatic metastasis and TNM stage (P=0.013 and P=0.023, respectively). However, no significant associations were detected between *H. pylori* infection status and sex, age, tumor size, location, general type, invasion, vascular invasion or pathological differentiation (P>0.05; Table III).

Association between HCMV infection and clinicopathological features of patients with GC. The analysis of HCMV infection and patient clinicopathological characteristics identified a significant negative association between HCMV infection status and lymphatic metastasis (P=0.002), as well as TNM stage (P=0.026). No significant associations were observed

tissues. HCMV infection was positive in 58 cases (43.3%), comprising 48 cases positive only in tumor tissues and

Table III. Clinicopathological features, *H. pylori* and HCMV infection in gastric tumors.

Clinicopathological feature	*H. pylori* Negative, n (%)	Positive, n (%)	P-value	HCMV Negative, n (%)	Positive, n (%)	P-value
Sex			0.661			0.869
Female	15 (25.0)	21 (28.4)		20 (26.3)	16 (27.6)	
Male	45 (75.0)	53 (71.6)		56 (73.7)	42 (72.4)	
Age[a], years			0.464			0.692
<68	27 (45.0)	38 (51.4)		38 (50.0)	27 (46.6)	
≥68	33 (55.0)	36 (48.6)		38 (50.0)	31 (53.4)	
Tumor size, cm			0.177			0.992
<5	37 (61.7)	37 (50.0)		42 (55.3)	32 (55.2)	
≥5	23 (38.3)	37 (50.0)		34 (44.7)	26 (44.8)	
Tumor location			0.609[b]			0.579[b]
Antrum	39 (65.0)	44 (59.5)		46 (60.5)	37 (63.8)	
Corpus	12 (20.0)	12 (16.2)		15 (19.7)	9 (15.5)	
Cardia	8 (13.3)	15 (20.3)		14 (18.4)	9 (15.5)	
Diffuse	1 (1.7)	3 (4.1)		1 (1.3)	3 (5.2)	
General type			0.690			0.806
Non-ulcer	9 (15.0)	13 (17.6)		13 (17.1)	9 (15.5)	
Ulcer	51 (85.0)	61 (82.4)		63 (82.9)	49 (84.5)	
Tumor invasion			0.897			0.258
T1 + T2	16 (26.7)	19 (25.7)		17 (22.4)	18 (31.0)	
T3 + T4	44 (73.3)	55 (74.3)		59 (77.6)	40 (69.0)	
Lymphatic metastasis			0.013[c]			0.002[c]
N0 + N1	34 (56.7)	26 (35.1)		25 (32.9)	35 (60.3)	
N2 + N3	26 (43.3)	48 (64.9)		51 (67.1)	23 (39.7)	
TNM stage			0.023[c]			0.026[c]
I+II	32 (53.3)	25 (33.8)		26 (34.2)	31 (53.4)	
III+IV	28 (46.7)	49 (66.2)		50 (65.8)	27 (46.6)	
Vascular invasion			0.332			0.706
No	39 (65.0)	42 (56.8)		47 (61.8)	34 (58.6)	
Yes	21 (35.0)	32 (43.2)		29 (38.2)	24 (41.4)	
Differentiation			0.814[b]			0.293[b]
Well	3 (5.0)	3 (4.1)		3 (3.9)	3 (5.2)	
Moderate	8 (13.3)	14 (18.9)		9 (11.8)	13 (22.4)	
Poor	35 (58.3)	43 (58.1)		49 (64.5)	29 (50.0)	
Other	14 (23.3)	14 (18.9)		15 (19.7)	13 (22.4)	

[a]Divided into two groups based on median age; [b]Calculated using Fisher's exact test; [c]P<0.05. HCMV, human cytomegalovirus; *H. pylori*, *Helicobacter pylori*.

between HCMV infection status and sex, age, tumor size, location, general type, invasion, vascular invasion or tumor differentiation (P>0.05; Table III).

Clinical features of HCMV and H. pylori co-infection in patients with GC. The 134 patients were divided into HCMV[+] and HCMV[-] groups. In these groups, the patients were subdivided according to the presence or absence of *H. pylori*. Subgroup analysis revealed that in the HCMV[+] group, *H. pylori* infection was not significantly associated with

lymphatic metastasis (P=0.408), whereas in the HCMV[-] group, *H. pylori* infection was significantly associated with advanced lymphatic metastasis (P=0.003) and TNM stage (P=0.023). Tumor invasion exhibited no statistical significances between the two groups (Table IV).

Additionally, the 134 patients were divided into *H. pylori*[+] and *H. pylori*[-] groups, which were further divided according to HCMV infection status. Subgroup analysis demonstrated that HCMV infection status was negatively associated with lymphatic metastasis (P<0.001) and TNM stage (P=0.026)

Table IV. Association of *H. pylori* and HCMV co-infection status with clinicopathological features.

Clinicopathological feature	HCMV+ *H. pylori*+/-	P-value	HCMV- *H. pylori*+/-	P-value	*H. pylori*+ HCMV+/-	P-value	*H. pylori*- HCMV+/-	P-value
Tumor invasion		0.751		0.977		0.498		0.340
T1 + T2	10/8		9/8		10/9		8/8	
T3 + T4	24/16		31/28		24/31		16/28	
Lymphatic metastasis		0.408		0.003[a]		<0.001[a]		0.202
N0 + N1	19/16		7/18		19/7		16/18	
N2 + N3	15/8		33/18		33/15		8/18	
TNM stage		0.246		0.023[a]		0.026[a]		0.245
I+II	16/15		9/17		16/9		15/17	
III+IV	18/9		31/19		18/31		9/19	

[a]P<0.05. HCMV, human cytomegalovirus; *H. pylori*, *Helicobacter pylori*.

in the *H. pylori*+ group. In the *H. pylori*- group, there were no significant associations between HCMV infection status and tumor invasion, lymphatic metastasis or TNM stage (Table IV).

Univariate and multivariate analyses based on lymphatic metastasis. The association between infection status and lymphatic metastasis was analyzed using univariate and multivariate analyses. Univariate analysis revealed that *H. pylori* (P=0.013), HCMV (P=0.002), tumor size (P<0.001), tumor invasion (P<0.001) and vascular invasion (P<0.001) were significantly associated with lymphatic metastasis (Table V). Other features exhibited no statistical significance (P>0.05). Additionally, *H. pylori* [P=0.007; odds ratio (OR)=3.51], tumor invasion (P=0.012; OR=4.31) and vessel invasion (P=0.004; OR=3.93) were independent risk factors for lymphatic metastasis, whereas HCMV (P=0.001; OR=0.21) was an independent protective factor for lymphatic metastasis, according to the multivariate logistic regression analysis (Table V).

To further clarify the association between lymphatic metastasis and co-infection, the patients were divided into HCMV-/*H. pylori*-, HCMV+/*H. pylori*-, HCMV-/*H. pylori*+ and HCMV+/*H. pylori*+ groups. Univariate analysis revealed a significant association between lymphatic metastasis and different co-infection statuses (P=0.001). Compared with the HCMV-/*H. pylori*- group, neither HCMV+/*H. pylori*- (P=0.205) nor HCMV+/*H. pylori*+ (P=0.622) were associated with lymphatic metastasis, whereas the HCMV-/*H. pylori*+ group (P=0.004) was a significant risk factor for lymphatic metastasis. Multivariate analysis revealed that HCMV-/*H. pylori*+ status was independently associated with lymphatic metastasis (P=0.005; OR=6.00; Table VI).

Discussion

PCR is the most widely used test in the laboratory to detect *H. pylori* infection in gastric tissues, and its positive rate is consistent with that of histopathological methods in fresh GC tissues (21). In the present study, the *H. pylori* infection rate detected using PCR was 55.2%, which was similar to the results of a previous study (22).

H. pylori is a pathogen recognized as a group I carcinogen by the International Agency for Research on Cancer (3). *H. pylori* infection is considered to progress to gastric malignancy via two different mechanisms (22,23). First, the virulence factors produced by *H. pylori*, such as cytotoxin-associated gene A, directly damage the mucosa, resulting in neoplastic transformation (23). Second, *H. pylori* causes chronic inflammation; infiltration of neutrophils and lymphocytes upregulate numerous pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor α and inflammation-associated transcription factors such as NF-κB (22). These inflammation-associated factors, particularly NF-κB and IL-8, are important mediators in gastric carcinogenesis (22). *H. pylori* infection and consequent chronic inflammation are considered to be a major step in the occurrence and development of GC (22). In the present study, analysis of the association between *H. pylori* infection and clinicopathological features revealed that the *H. pylori*+ group exhibited a higher rate of regional lymph node metastasis and a higher TNM stage. Additionally, multivariate analysis revealed that *H. pylori* infection was independently associated with lymphatic metastasis.

The study of *H. pylori* has long been the focus of GC research, although most studies (3,20,22,23) have focused on *H. pylori* itself, ignoring the complicated pathogenic microbiological environment of GC and neglecting the synergistic or antagonistic effects of *H. pylori* with other pathogenic microorganisms. Increasing evidence has suggested that HCMV is detected at a very low level in various types of tumor (12,24,25). For example, Cobbs *et al* (24) have detected HCMV genes and their products in glioblastoma multiforme (GBM). In the present study, HCMV was detected in 43.3% of patients with GC, and compared with our previous GC study (15), the results obtained using different primers exhibited improved consistency. This discrepancy may be due to improved

Table V. Univariate and multivariate logistic regression analyses of lymphatic metastasis and infection status.

Clinicopathological feature	N0 + N1	N2 + N3	Univariate analysis		Multivariate analysis	
			OR (95% CI)	P-value	OR (95% CI)	P-value
H. pylori				0.013[a]		0.007[a]
No	34	26	1		1	
Yes	26	48	2.41 (1.20, 4.86)		3.51 (1.41, 8.71)	
HCMV				0.002[a]		0.001[a]
No	35	23	1		1	
Yes	25	51	0.32 (0.16, 0.66)		0.21 (0.08, 0.53)	
Sex				0.730		0.759
Female	17	19	1		1	
Male	43	55	1.14 (0.53, 2.46)		1.17 (0.42, 3.25)	
Age[b], years				0.314		0.825
<68	32	33	1		1	
≥68	28	41	1.42 (0.72, 2.81)		1.11 (0.45, 2.69)	
Tumor size, cm				<0.001[a]		0.060
<5	44	30	1		1	
≥5	16	44	4.03 (1.93, 8.43)		2.44 (0.96, 6.16)	
Tumor location			1.37 (0.91, 2.06)	0.543[c]	1.12 (0.67, 1.85)	0.669
Antrum	41	42				
Corpus	10	14				
Cardia	8	15				
Diffuse	1	3				
General type				0.385		0.304
Non-ulcer	8	14	1		1	
Ulcer	52	60	0.66 (0.26, 1.70)		0.49 (0.12, 1.93)	
Tumor invasion				<0.001[a]		0.012[a]
T1 + T2	25	10	1		1	
T3 + T4	35	64	4.57 (1.97, 10.60)		4.31 (1.38, 13.40)	
Vessel invasion				<0.001[a]		0.004[a]
No	47	34	1		1	
Yes	13	40	4.25 (1.98, 9.15)		3.93 (1.55, 9.96)	
Differentiation				0.160		0.221
Well/moderate	17	11	1		1	
Poor	32	46	2.22 (0.92, 5.37)		2.25 (0.73, 6.92)	
Other	11	17	2.39 (0.82, 6.98)		3.24 (0.79, 13.22)	

[a]P<0.05; [b]Divided into two groups based on median age; [c]Calculated using Fisher's exact test. N, lymphatic metastasis; OR, odds ratio; HCMV, human cytomegalovirus; *H. pylori*, *Helicobacter pylori*.

specimen quality control, optimization of PCR conditions and quality of primers. The HCMV+ specimens were mostly tumor tissues, which was consistent with studies performed in prostatic carcinoma, colorectal cancer and malignant glioma (12,24,25). Similarly to previously reported PCR results (26), the detection rate of *H. pylori* in peri-tumoral tissues was higher than that in tumor tissues in the present study, suggesting that *H. pylori* and HCMV infections do not completely overlap spatially.

Rahbar *et al* (27) have demonstrated the prognostic value of the infection status of HCMV in patients with GBM. In breast cancer, HCMV proteins have been detected in sentinel lymph nodes, suggesting that HCMV may contribute to metastasis (11). A number of genes encoded by HCMV, such as UL123, UL36-38, UL97 and US28, participate in cellular transformation (28). In addition, several HCMV genes serve roles in apoptosis, cell cycle, invasion and migration of cancer cells, and angiogenesis in glioblastoma (17,28-37) Furthermore, HCMV participates in tumor immunomodulation (38,39). In the present study, statistical analysis revealed that lymphatic metastasis and TNM stage were associated with a negative HCMV status.

Table VI. Univariate and multivariate logistic regression analyses of lymphatic metastasis and co-infection status.

Clinicopathological feature	N0 + N1	N2 + N3	Univariate analysis		Multivariate analysis	
			OR (95% CI)	P-value	OR (95% CI)	P-value
Infection status				0.001[a]		
HCMV⁻/*H. pylori*⁻	10	12	1		1	
HCMV⁺/*H. pylori*⁻	9	6	0.50 (0.17, 1.46)	0.205[b]	0.41 (0.11, 1.56)	0.192[b]
HCMV⁺/*H. pylori*⁺	9	11	0.79 (0.31, 2.02)	0.622[b]	0.75 (0.23, 2.41)	0.624[b]
HCMV⁻/*H. pylori*⁺	4	20	4.71 (1.66, 13.40)	0.004[a,b]	6.00 (1.71, 21.04)	0.005[a,b]

[a]$P<0.05$; [b]χ^2 test vs. the HCMV⁻/*H. pylori*⁻ group. OR, odds ratio; HCMV, human cytomegalovirus; *H. pylori*, *Helicobacter pylori*.

HCMV causes chronic inflammation similarly to other tumor-associated pathogens, such as *H. pylori* (40). A previous study has reported the potential interaction between HCMV and other pathogens (41). Compared with HCMV⁻/human papilloma virus (HPV)⁻ cases, HCMV⁺/HPV⁺ but not HCMV⁻/HPV⁺ patients with cervical carcinoma had a significantly higher rate of metastasis to lymph nodes, indicating a synergetic effect between HCMV and HPV on lymphatic metastasis in cervical carcinoma (41). In the present study, analysis of the clinical significance of HCMV and *H. pylori* co-infection revealed that in the HCMV⁺ group, *H. pylori* infection status was not associated with lymphatic metastasis. However, in the HCMV⁻ group, *H. pylori* infection status was significantly associated with lymphatic metastasis. In addition, the co-infection status was associated with lymphatic metastasis, and compared with the HCMV⁻/*H. pylori*⁻ group, the HCMV⁻/*H. pylori*⁺ status was a potential risk factor for lymphatic metastasis. Multivariate analysis revealed that the HCMV⁻/*H. pylori*⁺ status was independently associated with high level of lymphatic metastasis. Therefore, HCMV and *H. pylori* may interact in GC by exerting an antagonistic effect on lymphatic metastasis progression. These contradictory results in GC and cervical carcinoma may be associated with the complexity of the GC microenvironment and with HCMV infection itself. A recent study revealed no association between HCMV infection and lymphatic metastasis, nor the characteristics of co-infection with *H. pylori* (26). However, 65% of samples in the aforementioned study were located at the proximal position of the stomach, in contrast to the cohort in the present study in which the majority (62%) of tumors were located at the distal position. This suggests that the role of HCMV may be associated with the tumor location in the stomach. In human malignant glioma, which is the most studied HCMV-associated tumor, HCMV infection is mainly regarded as a tumor promoter (42,43). However, previous studies have revealed that HCMV may be a protective factor in the outcome of gastrointestinal tumors, breast cancer and hepatocellular carcinoma (44-47). Furthermore, the HCMV genes US28 (48) and UL123 (49) can accelerate tumor growth and enhance cancer cell stemless, while UL138 can promote the apoptosis of cancer cells (17). A recent report has revealed that in GC, low β-catenin-interacting protein 1 (CTNNBIP1) expression is associated with well-differentiated tumor grades, and CTNNBIP1 downregulation is significantly associated with HCMV infection (50), indicating a possible tumor inhibition mechanism of HCMV. The Epstein-Barr virus (EBV), which is a cancer promoter in Burkitt lymphoma and nasopharyngeal carcinoma, may also serve a protective role in GC (51-54). Several studies have demonstrated that EBV-associated GC exhibits a lower rate of lymph node metastasis (53,54). These results suggest that HCMV may serve multiple roles in different types of tumor, which may be associated with its latent and proliferative status, varied tumor microenvironment, interaction with other tumor-associated pathogens, diverse immunomodulatory effects of HCMV and HCMV gene expression characteristics (51-54).

Based on the results of the present study, it can be speculated that there may be a pathway through which HCMV inhibits *H. pylori*⁺ GC lymphatic metastasis, which may be used as a guide for clinicians when planning the patients' treatment modalities. The research on *H. pylori* mainly focuses on its carcinogenic role, since *H. pylori* is a recognized cause of GC. Eradication of *H. pylori* is an effective way to prevent GC. However, to the best of our knowledge, there is no conclusive evidence that patients with GC can benefit from eradication or non-eradication of *H. pylori*. The present results may suggest that patients with GC who are positive for both *H. pylori* and HCMV may be exempted from *H. pylori* eradication.

There were certain limitations in the current study. Only the positive rates of *H. pylori* and HCMV infection were detected, without their copy numbers, so the role of the infection level in the co-infection remains to be explored. Additionally, only PCR was used as a detection method, not supplemented by immunohistochemistry, *in situ* hybridization or other methods, which may affect the true positive rate of the infections.

In conclusion, the results of the present study revealed that *H. pylori* infection may increase tumor aggressiveness by promoting lymphatic metastasis. However, in patients with HCMV and *H. pylori* co-infection, *H. pylori* may lose its tumor-promoting ability, and this group of patients exhibited a lower risk of developing a high level of lymphatic metastasis. This suggests that HCMV may inhibit lymphatic metastasis through an *H. pylori*-associated pathway, which should be further investigated in future research.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CC and SC performed the experiments, analyzed and interpreted the data, and drafted the manuscript. ZH collected the clinical data and performed statistical analysis. WX designed the study and performed the experiments. TZ, CM, LZ and XSu collected the tissue specimens. XSh analyzed and interpreted the data, revised the manuscript and finally approved the version of the manuscript for publication. TK and XX contributed to the conception and design of the study and interpreted the data; in addition, XX supervised the study, analyzed the data, provided the project funding, revised the manuscript and finally approved the version of the manuscript for publication. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Human Research Ethics Committee of the Second Affiliated Hospital of Wenzhou Medical University (Wenzhou, China), and written informed consent was provided by all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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