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Original contribution

The implication of tumor-infiltrating lymphocytes in Epstein-Barr virus–associated gastric carcinoma☆,☆☆

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Summary Epstein-Barr virus (EBV)–associated gastric carcinoma (EBVaGC) is a distinct entity that has conspicuously inflammatory infiltration compared with EBV-negative gastric carcinoma. To date, the local immune status in EBVaGC and its relationship with patient prognosis and apoptosis of tumor cells are largely unknown. In this study, we evaluated the density of different types of tumor-infiltrating lymphocytes (TILs) in 53 EBVaGCs and 67 EBV-negative gastric carcinomas and analyzed its relationship with patient outcomes and apoptosis of tumor cells in EBVaGC. The average number of CD3+ total T cells, CD8+ T cells, CD79α+ B cells, CD56+ natural killer cells, Fascin+ dendritic cells (DCs), and FoxP3+ Tregs and the average proportions of Ki-67, interleukin 1β, granzyme B, interferon γ, and interleukin 10 in TILs were higher in EBVaGC, and CD8+ T cells were the predominant constituent cells of TILs in EBVaGC. Patients with higher numbers of CD3+ total T cells, CD8+ T cells, CD79α+ B cells, and Fascin+ DCs survived longer in EBVaGC, and CD8+ T cells and Fascin+ DCs were independent prognostic factors for patient survival. Besides, CD8+ T cells were positively correlated with apoptotic index of tumor cells. However, the apoptosis of tumor cells was lower, and the expression of survivin and NF-κBp65 in tumor cells was upregulated in EBVaGC. These findings suggested that CD3+ total T cells, CD8+ T cells, CD79α+ B cells, and Fascin+ DCs predict a better prognosis in EBVaGC; CD8+ T cells might through a nonapoptotic pathway eliminate tumor cells, thereby improving the patient prognosis.

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1. Introduction

Epstein-Barr virus (EBV) is a ubiquitous human herpes DNA virus that establishes a lifelong persistent infection in more than 90% of population worldwide [1]. It is implicated in the etiology of many human malignancies, including a variety of lymphoid malignancies such as Hodgkin lymphoma and Burkitt lymphoma, as well as some epithelial carcinomas such as nasopharyngeal carcinoma and a subset of gastric carcinoma (GC) [2]. EBV-encoded RNAs could be detected by in situ hybridization in nearly 10% of GC, which are defined as EBV-associated GC (EBVaGC) [3]. Recently, The Cancer Genome Atlas research network divided gastric adenocarcinoma into 4 subtypes according to comprehensive molecular characterization and proposed that EBVaGC is 1 of the 4 subtypes [4]. Compared with EBV-negative GC (EBVnGC), EBVaGC has specific histologic features that show irregularly anastomosing tubules accompanied with lots of lymphocytic infiltration and result in a "lace-like" pattern at low magnification; thus, plenty of lymphocytes accompanied with rich cytokines infiltration in the tumor microenvironment are the most remarkable features [5].

Tumor-infiltrating lymphocytes (TILs) constitute the main cellular component of the immune-active tumor microenvironment, which are represented by T cells, B cells, natural killer cells (NKs), and dendtritic cells (DCs) [6]. Extensive studies have proved that there are 3 main pathways involved in TIL-mediated tumor cell death, namely, granule exocytosis (perforin and granzyme B [GrB]), death ligands (FasL/Fas), and cytotoxic T lymphocyte–secreted cytokines (tumor necrosis factor α [TNF-α] and interferon γ [IFN-γ]), and those are considered a representation of the host immune activation response [7,8]. However, tipping the balance of immunosurveillance from tumor elimination to tumor promotion seems to be a complex process under the common regulation of TIL-mediated activation of immunity and tumor-induced tolerance of T cells [9]. Several studies have demonstrated that the tolerance of T cells mainly depended on the immunosuppressive functions of FoxP3+ Tregs and cytokine interleukin (IL) 10 [10,11]. The ratio of CD4/CD8 has been used as a clinically index to evaluate patients' immune level, and the higher ratio means stronger levels of immunity [12].

Besides, decades of research have shown that a large number of TIL infiltration predict a better prognosis in various human malignances [13-15]. Recently, a clinical study showed that TILs could contribute to the improved survival of patients with GC [16]. EBVaGC has been described as an individual entity with conspicuously lymphocyte infiltration and possesses a better prognosis [5]. Nevertheless, very few studies have reported on the immune status of microenvironment in EBVaGC and prognostic effect of the density of TILs in patients with EBVaGC.

In this study, by means of immunohistochemistry (IHC), we comprehensively investigated the density of different types of TILs in EBVaGC and EBVnGC tissues. Specific cytokines that are associated with immunoactivation and immunosuppression were evaluated to assess the immune status of tumor microenvironment. Furthermore, the prognostic effects of different types of TILs were analyzed in EBVaGC. In addition, we also preliminarily compared the difference of TILs and apoptosis of tumor cells between conventional GC (CGC) and gastric remnant carcinoma EBVaGC (GRC) in EBVaGC.

2. Materials and methods

2.1. Patient samples

Forty-five (6.7%) of 676 consecutive CGC cases and 8 (30.8%) of 26 GRC cases were identified as EBVaGCs by EBV-encoded RNA 1 in situ hybridization [17]. The cases were collected from the Second and Third Affiliated Hospitals of Sun Yat-sen University and the Guangzhou First Municipal People's Hospital between January 1, 2000, and December 31, 2006. In the present study, EBVaGCs (n = 45) and matched EBVnGCs (n = 49) in CGC, as well as EBVaGCs (n = 8) and EBVnGCs (n = 18) in GRC were enrolled. All patients were restaged according to the seventh American Joint Committee on Cancer TNM staging system for gastric cancer [18], and the clinicopathological characteristics of EBVaGC and EBVnGC in CGC and GRC are summarized in Supplementary Table S1. Informed consents were obtained from all individuals whose samples were analyzed in the present study, and ethical guidelines under the Declaration of Helsinki were followed. The research protocol was approved by the Clinical Research Ethics Committee of the Third Affiliated Hospital, Sun Yat-sen University.

2.2. IHC staining

The 2-step EnVision IHC procedure (Dako, Glostrup, Denmark) was carried out on the 4-μm-thickness sections of paraffin-embedded tissues. Briefly, the tissue sections were dewaxed in xylene, rehydrated in gradient ethanol solutions, and then heated for antigen retrieval. Endogenous peroxidase activity was blocked with hydrogen peroxide. Sections were incubated with different primary antibodies at 4°C overnight, followed by incubation with secondary antibody for 30 minutes. Immunostaining was visualized with 3,3′-diaminobenzidine, and the slides were counterstained with hematoxylin. Slides with known positive reactivity were used as positive controls, and phosphate-buffered saline instead of specific primary antibodies was used as a negative control. The primary antibodies used in this study and their retrieval methods, as well as the dilutions, are listed in Supplementary Table S2.

The coexpression of Ki-67 and CD8 was detected by sequential double-immunohistochemical staining according to the instructions of the double-staining DouSP Double Stain System (Maixin, Fuzhou, China).

2.3. IHC scoring

All slides were quantitatively evaluated under conventional light microscopy by 2 investigators. Density of the infiltrating immune cells (CD3+ total T cells, CD4+ T cells, CD8+ T cells, CD79α+ B cells, Fascin+ DCs, and FoxP3+ Tregs) was determined by counting the positively stained immune cells in 15 randomly high-power microscopic fields (magnification ×400, high-power field [HPF]) around the tumor nests and calculating the mean number of positively stained cells per HPF [19]. For CD56+ NK cells, the number of positively stained cells was counted in 25 randomly middle-power microscopic fields (magnification ×200, middle-power field [MPF]), and the mean number of positively stained cells per MPF was calculated [20]. For Ki-67, at least 1000 CD8+ T cells (red cytomembrane staining) in 15 randomly HPF around the tumor nests were counted, and the percentage of Ki-67–positive CD8+ T cells (dark-blue nuclear staining) was calculated [21]. GrB, FasL, IL-1β, IFN-γ, TNF-α, and IL-10 in TILs were quantified as the percentage of positive cells in relation to the total number of TILs, by counting at least 200 TILs in 15 randomly HPF around the tumor nests [22]. The following weighted scoring method was adopted to quantify the survivin and NF-κBp65 expression. The mean percentages of positive tumor cells were determined in at least 5 randomly HPF and assigned to one of the following categories: (*a*) 0, ≤5%; (*b*) 1, 6% to 25%; (*c*) 2, 26% to 50%; and (*d*) 3, >50%; The percentage of positive tumor cells and the staining intensity scores were multiplied to produce a weighted score for each case: 0 to 1, (−); 2 to 3,(+); 4 to 6, (++); and >6, (+++) [23].

2.4. TUNEL assay

Apoptotic tumor cells were analyzed by terminal deoxynucleotidyl transferase–mediated dUTP-biotin nick end-labeling (TUNEL) using a terminal deoxynucleotidyl transferase–FragEL DNA Fragmentation Detection Kit (Calbiochem, Darmstadt, Germany). Apoptosis index (AI) was determined as the percentage of TUNEL-positive carcinoma cells in comparison with the total number of carcinoma cells in each specimen. At least 1000 tumor cells from 10 randomly selected fields were quantified (magnification ×400, HPF) [24].

2.5. Statistical analysis

For all immunohistochemical markers, the median values were used as cutoff values for definition of subgroups. For AI, the mean value was used as the cutoff value. Overall survival (OS) was calculated from the date of surgery to the date of death from any cause or the last day of follow-up. The log-rank test was used to perform univariate analyses, and the survival curves were constructed using the Kaplan-Meier method. Multivariate analysis of putative prognostic factors was performed using a Cox proportional hazards model. Correlations were evaluated using the Spearman rank correlation coefficient. Two-tailed $P < .05$ was considered statistically significant.

Table 1 Expression of TILs and cytokines in EBVaGC and EBVnGC in CGC and GRC

	CGC			GRC		
	EBVaGC (n = 45)	EBVnGC (n = 49)	*P*	EBVaGC (n = 8)	EBVnGC (n = 18)	*P*
CD3	83.31±36.66	28.65±18.17	<.001	100.38±34.29	56.50±40.40	.017
CD4	23.04±16.79	21.00±18.04	NS	8.37±5.78	18.28±13.20	.048
CD8	60.56±33.86	18.80±15.53	<.001	71.00±34.61	32.39±14.54	.007
CD4/CD8	0.77±1.34	1.79±1.80	NS	0.16±0.12	0.71±0.68	.005
CD79α	21.98±17.61	9.20±5.63	.002	34.25±13.57	14.83±11.07	.014
CD56 [a]	3.40±2.26	1.63±1.13	.042	2.91±1.63	1.04±0.82	.008
Fascin	18.98±9.67	12.45±9.36	.008	23.38±9.26	16.33±6.49	.035
FoxP3	16.04±12.36	9.31±7.07	.038	16.90±11.22	9.62±6.89	.028
GrB (%) [b]	30.58±22.68	13.40±11.85	.004	34.38±21.62	16.72±13.96	.027
FasL (%) [b]	29.53±20.67	26.65±16.31	NS	30.00±15.58	21.11±17.62	NS
TNF-α (%) [b]	24.49±21.73	18.35±16.75	NS	33.75±24.60	18.35±16.75	NS
IFN-γ (%) [b]	43.67±27.71	16.00±14.44	<.001	39.38±15.68	31.67±27.22	.048
Ki-67 (%) [c]	22.51±11.22	12.05±11.49	.001	29.00±14.91	9.56±7.22	.006
IL-1β (%) [b]	21.58±19.09	11.75±9.03	.037	30.63±20.78	12.17±10.44	.003
IL-10 (%) [b]	20.89±17.87	3.25±2.20	.003	27.50±22.88	10.94±8.04	.040

NOTE. The variables in the table represent average number of positive cells per HPF (mean ± SD, ×400 a ;).
Abbreviation: NS, no statistical significance ($P > .05$).
[a] The average number of positive cells per MPF (mean ± SD, ×200 ;).
[b] The average positive proportion in TIL per HPF (mean ± SD, ×400);.
[c] The average positive proportion in CD8+ TIL per HPF (mean ± SD, ×400).

All statistical analyses were performed using SPSS 20.0 statistics software (SPSS, Chicago, IL).

3. Results

3.1. The density of different types of TILs in EBVaGC

The average numbers of CD3+ total T cells, CD79α+ B cells, CD56+ NK cells, and Fascin+ DCs were all significantly higher in EBVaGC than those in EBVnGC (all $P < .05$; Table 1 ; and Fig. 1). As described in Table 1, TILs were mainly composed of CD8+ T cells, and the density of CD8+ T cells in EBVaGC was more higher than that in EBVnGC (60.56 ± 33.86/HPF versus 18.80 ± 15.53/HPF, $P < .001$), whereas the number of CD4+ T cells was similar between EBVaGC and EBVnGC (23.04 ± 16.79/HPF versus 21.00 ± 18.04/HPF, $P > .05$). In addition, the CD4/CD8 ratio was significantly lower in EBVaGC (0.77 ± 1.34 versus 1.79 ± 1.80, $P = .005$).

Besides, we also analyzed the profiling of TILs in both EBVaGC and EBVnGC according to the degree of

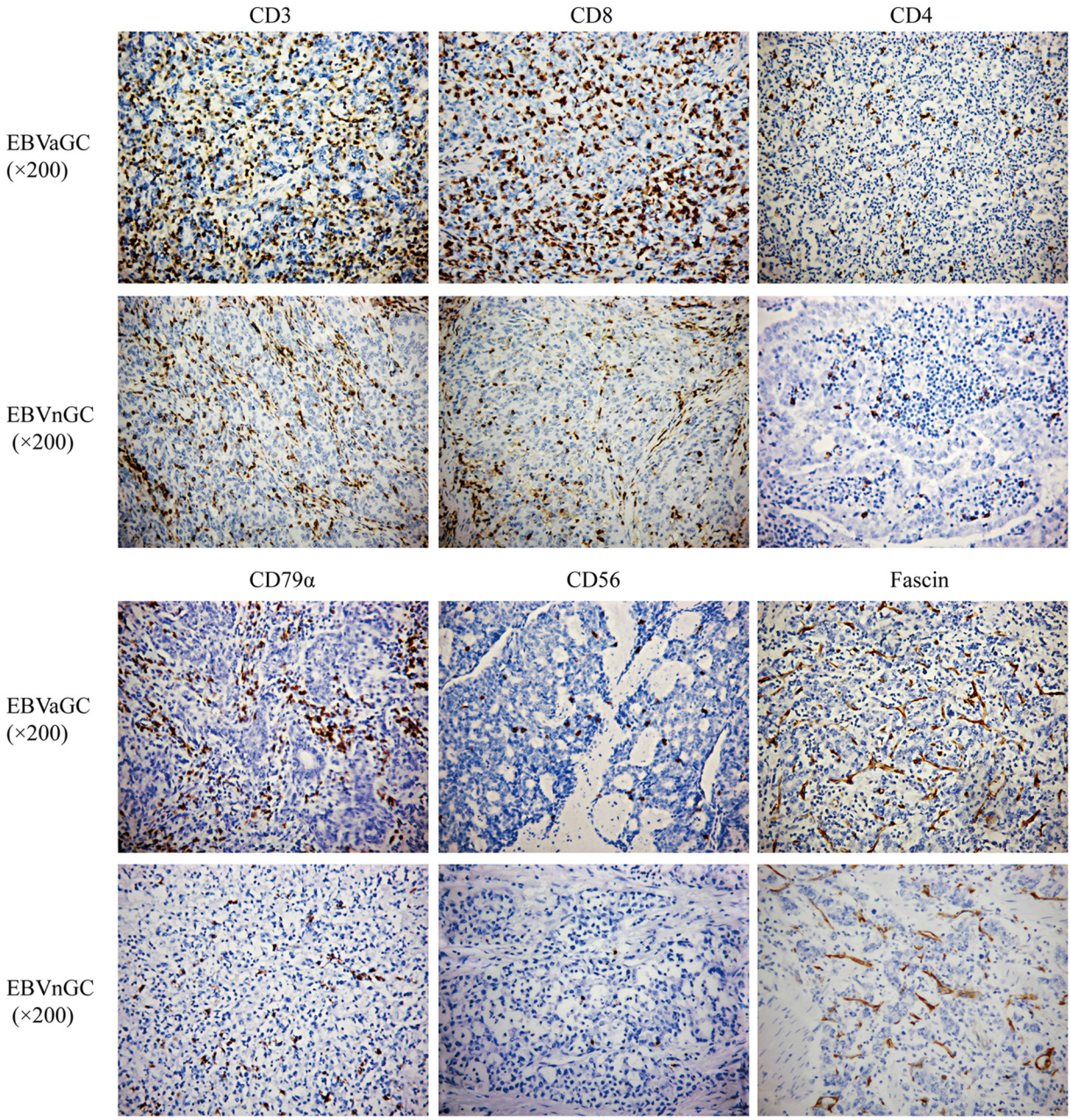

Fig. 1 The density of different types of TILs in EBVaGC. Representative images showed the higher number of CD3+, CD8+, CD79α+, CD56+, and Fascin+ cells in EBVaGC compared with those in EBVnGC. CD4 showed no statistically significant difference between EBVaGC and EBVnGC (original magnification ×200).

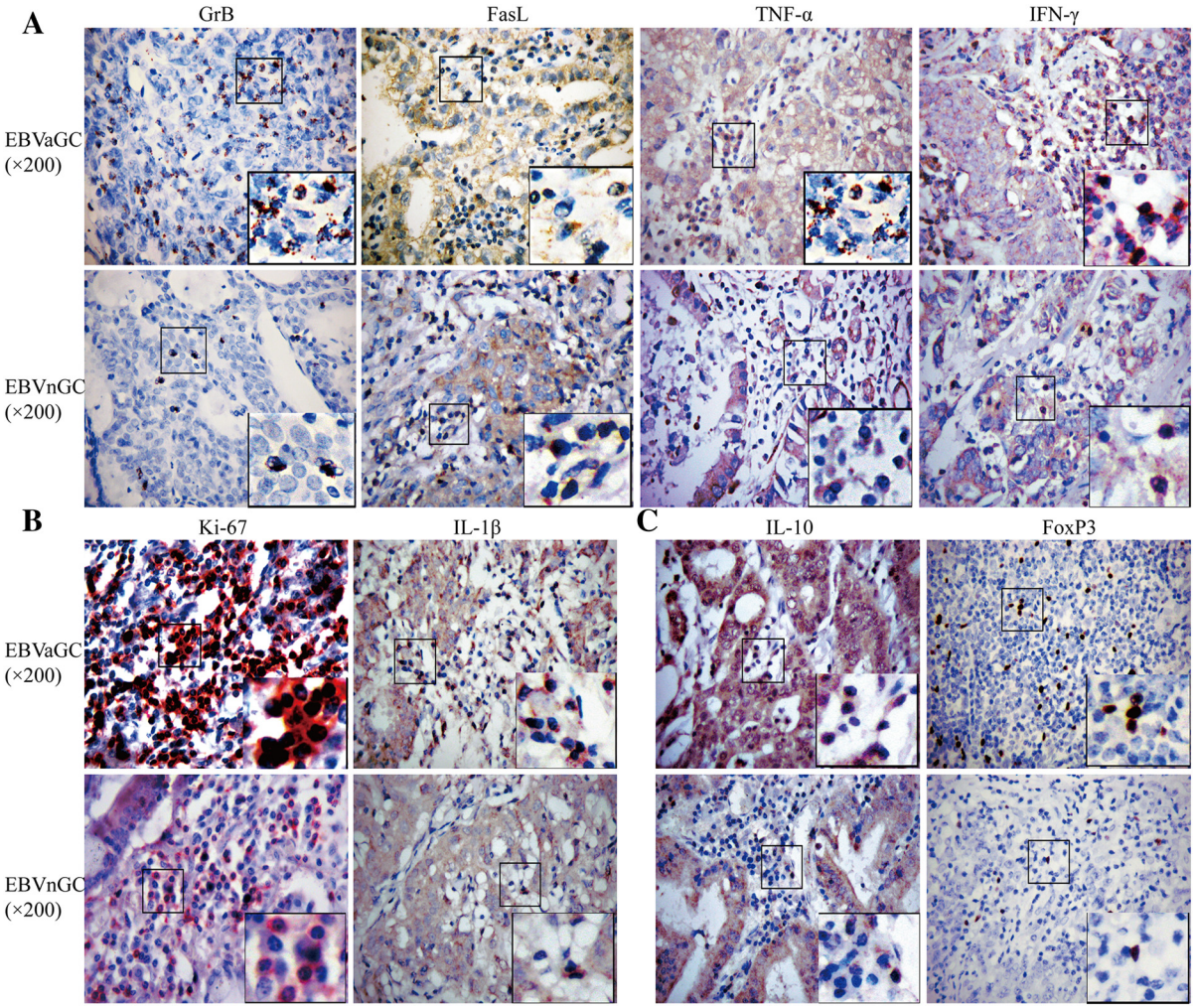

Fig. 2 Immunoactivation and immunosuppression coexisted in EBVaGC. A, Representative cases showed the higher positive proportions of GrB and IFN-γ in TILs in EBVaGC compared with EBVnGC. B, Representative cases showed the higher positive proportions of Ki-67 and IL-1β in TILs. The left column showed CD8/Ki-67 double-immunohistochemical-staining results. Ki-67 was located in nuclei and stained black, whereas CD8 was expressed in lymphocyte membrane and stained red. C, Representative cases described the higher positive proportion of IL-10 and the higher number of FoxP3+ cells in TILs in EBVaGC contrasted with EBVnGC (original magnification ×200).

differentiation of the tumor cells. As described in Supplementary Table S3, we found that CD8+ T cells were significantly higher in the poorly differentiated/undifferentiated group than those in the well-/moderately differentiated group in EBVaGC (67.08 ± 33.91 versus 34.44 ± 17.71, $P = .007$). In EBVnGC, the CD4+ T cells were significantly higher in the poorly differentiated/undifferentiated group than those in the well-/moderately differentiated group (27.26 ± 13.86 versus 12.50 ±15.20, $P = .032$).

3.2. Immunoactivation and immunosuppression coexisted in EBVaGC

As presented in Fig. 2A, we analyzed the expression of GrB, FasL, TNF-α, and IFN-γ in TILs to assess the immunoactivation in tumor microenvironment. The average positive proportions of GrB and IFN-γ in TILs were higher in EBVaGC than those in EBVnGC ($P = .004$ and $P < .001$, respectively), whereas the expression of FasL and TNF-α in TILs showed no statistically significant difference between EBVaGC and EBVnGC ($P > .05$; Table 1). At the same time, we also explored the expression of Ki-67 and IL-1β to evaluate the TILs' proliferative activity and chemotaxis (Fig. 2B). The expression of Ki-67 and IL-1β in TILs also increased in EBVaGC ($P = .001$ and $P = .037$; respectively; Table 1).

On the other hand, we also studied the number of Tregs and IL-10 expression in TILs to evaluate the immunosuppression in tumor microenvironment. As described in Table 1 and Fig. 2C, the average number of FoxP3+ Tregs and the positive rate of IL-10 expression in TILs were significantly higher in EBVaGC ($P = .038$ and $P = .003$, respectively).

3.3. Relationships between TILs and patient outcomes

As for survival, 32 cases of EBVaGC had survival data, whereas 13 cases of EBVaGC were lost to follow-up. For univariate OS analysis, CD3+ total T cells, CD8+ T cells, CD79α+ B cells, and Fascin+ DCs were potential prognostic factors for OS in EBVaGC (Supplementary Table S4). The higher number of CD3+ total T lymphocytes (median, 43 versus 16 months; $P = .0397$; Fig. 3A), CD8+ T lymphocytes (median, 43 versus 21 months; $P = .0395$; Fig. 3B), CD79α+ B lymphocytes (median, 28 versus 13 months; $P = .0446$; Fig. 3C), and Fascin+ DCs (median, 43 versus 18 months; $P = .0076$; Fig. 3D) predicted a significantly better OS. Furthermore, the multivariate analysis confirmed that CD8+ T cells and Fascin+ DCs were independent prognostic factors for EBVaGC's survival ($P = .007$ and $P = .040$, respectively; Supplementary Table S5).

3.4. Apoptosis of tumor cells and its correlations with TILs

The AI of tumor cells in EBVaGC was significantly lower than that in EBVnGC (0.82 ± 0.66 versus 1.18 ± 0.84, $P = .009$; Fig. 4A). Hino et al [25] have reported that EBV could use its latent protein, LMP2A, to activate the NF-κB–survivin pathway to rescue EBV-infected epithelial cells from apoptosis. Hence, we studied the expression of survivin and NF-κBp65 in tumor cells (Fig. 4B); as expected, higher expressions of survivin and NF-κBp65 in tumor cells were found in EBVaGC ($P = .006$ and $P = .004$, respectively; Table 2).

As described in Fig. 4C, we discovered that AI of tumor cells was positively correlated with CD8+ and GrB+ TILs in EBVaGC ($P = .002$ and $P < .001$, respectively).

3.5. The comparison of TILs, cytokines, and AI between CGC and GRC in EBVaGC or EBVnGC

The local immune status of EBVaGC in GRC was generally similar to that of EBVaGC in CGC (Table 1). Nevertheless, EBVaGC in GRC had more obvious immunosuppressive and antiapoptotic effect. EBVaGC in GRC had less CD4+ T cells ($P = .006$) and the lower CD4/CD8 ratio ($P = .012$; Table 3). The AI of EBVaGC tumor cells in

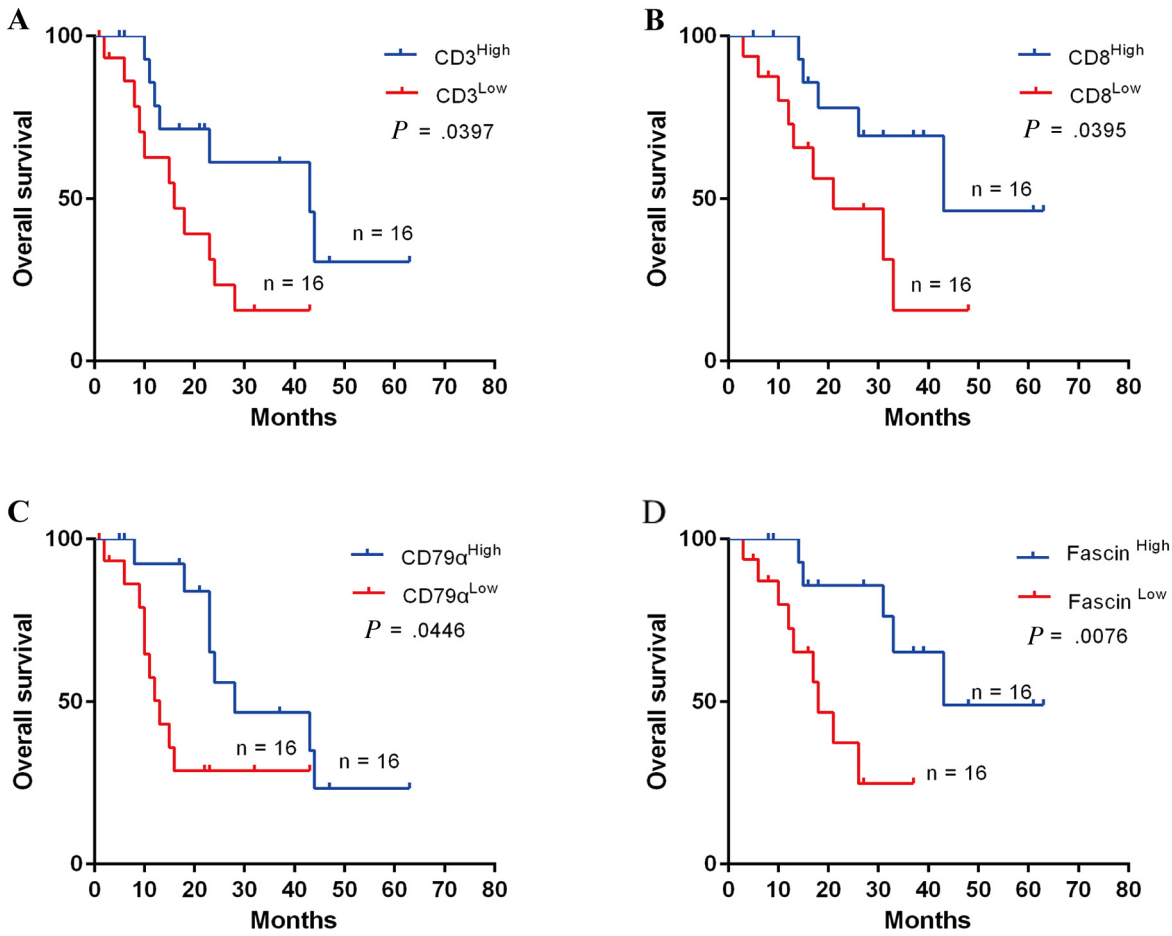

Fig. 3 Kaplan-Meier survival curves for OS in EBVaGC according to the density of CD3+ total T cells, CD8+ T cells, CD79α+ B cells, and Fascin+ DCs. Patients with higher number of CD3+ total T cells (A), CD8+ T cells (B), CD79α+ B cells (C), and Fascin+ DCs (D) had better OS in EBVaGC.

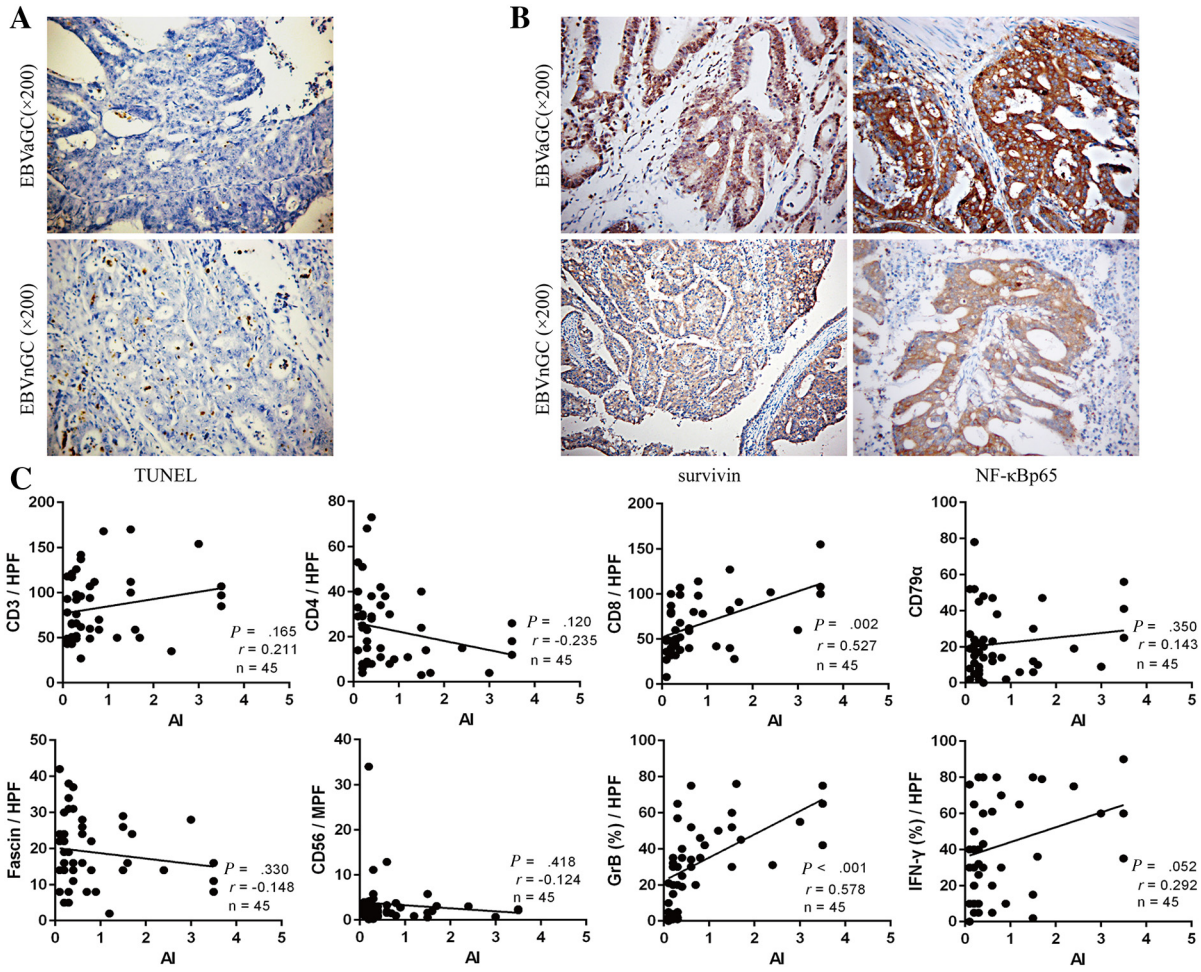

Fig. 4 Lower apoptosis rate of tumor cells and the correlations between AI and the TILs in EBVaGC. A, Apoptosis of tumor cells was detected by TUNEL assay showing densely packed with dark-brown particles in apoptotic tumor cell nucleus (original magnification ×200). B, Representative cases showed strong survivin and NF-κBp65 staining in EBVaGC and moderate staining in EBVnGC (original magnification ×200). C, Correlations between AI of tumor cells and TILs. AI of tumor cells was positively correlated with CD8+ and GrB+ TILs in EBVaGC.

GRC was lower than that in CGC, although the difference was not statistically significant ($P > .05$; Table 3). Notably, the expression of survivin in EBVaGC tumor cells in GRC was significantly higher than that in CGC ($P < .001$, Supplementary Table S6).

The profiling of TILs of EBVnGC was also different between CGC and GRC. EBVnGC in GRC had more obvious immunoactivation and immunosuppressive effect. EBVnGC in GRC had more CD8+ T cells ($P = .047$) and higher positive rate of IL-10 in TILs ($P = .002$) as well as the lower CD4/CD8 ratio ($P = .033$; Table 3).

Table 2 Survivin and NF-κBp65 expression in tumor cells in EBVaGC

Group	n	Survivin *				NF-κBp65 **			
		−	+	++	+++	−	+	++	+++
EBVaGC	45	18	10	14	3	5	12	19	9
EBVnGC	49	35	8	6	0	22	9	13	5

NOTE. P values were obtained using the Pearson χ^2 tests.
* $\chi^2 = 11.726$, $P = .006$.
** $\chi^2 = 13.254$, $P = .004$.

Table 3 The comparison of TILs, cytokines, and AI between CGC and GRC in EBVaGC or EBVnGC

	EBVaGC			EBVnGC		
	CGC (n = 45)	GRC (n = 8)	*P*	CGC (n = 49)	GRC (n = 18)	*P*
CD3	83.31±36.66	100.38±34.29	NS	28.65±18.17	56.50±40.40	.006 *
CD4	23.04±16.79	8.37±5.78	.006 *	21.00±18.04	18.28±13.20	NS
CD8	60.56±33.86	71.00±34.61	NS	18.80±15.53	32.39±14.54	.047 *
CD4/CD8	0.77±1.34	0.16±0.12	.012 *	1.79±1.80	0.71±0.68	.033 *
CD79α	21.98±17.61	34.25±13.57	.023 *	9.20±5.63	14.83±11.07	NS
CD56	3.40±2.26	2.91±1.63	NS	1.63±1.13	1.04±0.82	NS
Fascin	18.98±9.67	23.38±9.26	NS	12.45±9.36	16.33±6.49	NS
Foxp3	16.04±12.36	16.90±11.22	NS	9.31±7.07	9.62±6.89	NS
GrB	30.58±22.68	34.38±21.62	NS	13.40±11.85	16.72±13.96	NS
FasL	29.53±20.67	30.00±15.58	NS	26.65±16.31	21.11±17.62	NS
TNF-α	24.49±21.73	33.75±24.60	NS	18.35±16.75	18.35±16.75	NS
IFN-γ	43.67±27.71	39.38±15.68	NS	16.00±14.44	31.67±27.22	.007 *
Ki-67	22.51±11.22	29.00±14.91	NS	12.05±11.49	9.56±7.22	NS
IL-1β	21.58±19.09	30.63±20.78	NS	11.75±9.03	12.17±10.44	NS
IL-10	20.89±17.87	27.50±22.88	NS	3.25±2.20	10.94±8.04	.002 *
AI	0.82±0.66	0.65±0.53	NS	1.18± 0.84	1.58±1.14	NS

Abbreviation: NS, no statistical significance ($P > .05$).
* Indicates statistical significance.

4. Discussion

In this study, we evaluated the density of different types TILs and their impact on patient outcomes in EBVaGC. OS of patient with a higher density of CD3+ total T cells, CD8+ T cells, CD79α+ B cells, and Fascin+ DCs tended to be longer. Furthermore, CD8+ T cells were positively correlated with AI of tumor cells. These findings suggested that TILs play an important role in the survival of tumor cells, thereby impacting the patient prognosis in EBVaGC.

Recently, several studies proposed a dynamic and complex interaction between immune cells and tumor cells, the progression of carcinoma under the common regulation of TIL-mediated immunoactiviation, and tumor-induced immunosuppression [9,26]. In this study, the complex interaction was more obviously presented in EBVaGC; there are plenty of not only activated CD8+ T cells, NK cells, and DCs and their secreted antitumor cytokines GrB and IFN-γ but also a high density of FoxP3+ Tregs and immunosuppressive cytokines IL-10: hinting us that the stronger effect of immunoactiviation and immunosuppression were coexisted in EBVaGC tumor microenvironment.

Extensive studies have shown that tumor-infiltrating cells are associated with a better prognosis in various kinds of cancers, such as colorectal cancer [27], nasopharyngeal carcinoma [28], and GC [29]. Zitvogel and Kroemer [30] reported that the adaptive immunity mediated by T and B lymphocytes plays an effective antitumor response, and Anguille et al [31] demonstrated that the immunotherapeutic methods based on tumor-infiltrating DCs could prolong tumor patient's survival. A recent study on EBVaGC indicated that the total density of TILs could be a prognostic factor for predicting patient outcome [32]. However, the prognostic role of different types of TILs in patients of EBVaGC is largely unknown. In our findings, EBVaGC patients with higher density of CD3+ total T cells, CD8+ T cells, CD79α+ B cells, and Fascin+ DCs had better OS. Especially when the multivariate analysis was used, CD8+ T cells and Fascin+ DCs were discovered to be an independent prognostic factor for EBVaGC's survival.

Cytotoxic T cells and NK cells adopt the same basic mechanisms to eliminate cancer cells: one is by secreting ranule exocytosis (perforinand and GrB) and the other via the death ligand/death receptor. Both effector pathways trigger programmed intracellular events in cancer cells, leading to apoptotic cell death in most cases [33,34]. According to our results, CD8+ T cells and GrB+ TILs were positively correlated with AI of tumor cells in EBVaGC, and especially CD8+ T cells were the predominant constituent cells of TILs in EBVaGC. On the basis of these findings, we suspected that the infiltrating CD8+ T cells seem to eliminate cancer cells by inducing apoptosis of tumor cells, thereby impacting patient prognosis. Nevertheless, we found that the apoptosis of tumor cells in EBVaGC was less than that in EBVnGC. Previous study has concluded that EBV-encoded LMP2A could activate the NF-κB–survivin pathway to save EBV-infected epithelial cells from apoptosis [25]. Consistent with this finding, our result showed the higher expression of survivin and NF-κBp65 in EBVaGC tumor cells and indicated that EBVaGC cells survived from apoptosis probably through LMP2A-mediated activation of the NF-κB–survivin pathway. Among these, the stronger immunosuppression in EBVaGC may be one of the reasons contributing to the lower apoptosis of tumor cells. Besides, recent experimental evidence indicates that nonapoptotic cell death pathways that were induced via the inflammation caused by some GrB, IFN-γ, death ligands, and

proliferative effects of CD8 + T cells moderate the progression of carcinoma [8]. Together with our data, the high expression of Ki-67, GrB, and IFN-γ in CD8+ T cells in EBVaGC further pointed that the infiltrating CD8+ T cells eliminate tumor cells probably through nonapoptotic pathways, thereby improving patient prognosis.

It is well known that the frequency of EBVaGC in GRC was higher than that in CGC. Koriyama et al [35] have shown that the inflammation result of gastric mucosal damage plays an important role in the occurrence and development of EBVaGC. However, the difference of local immune status and apoptosis of tumor cells in EBVaGC between CGC and GRC is largely unknown. Here, we found that the EBVaGC in GRC had the stronger immunosuppression in tumor site and higher antiapoptosis effect, which may contribute to the development of EBVaGC in GRC, thereby leading to poorer prognosis.

These data should be interpreted cautiously. We investigated the function of TILs only by IHC. Thus, further large-scale studies and specific cell experiments on the function of TILs are needed to prove our results.

In conclusion, this study elucidated the prognostic values of different types of TILs and further predicted that CD8+ T cell and Fascin+ DC could be an independent prognostic indicator in EBVaGC. CD8+ T cells might through a nonapoptotic pathway eliminate tumor cells, thereby improving the patient prognosis.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.11.002.

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