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ORIGINAL ARTICLE

Expression of costimulatory molecules B7-H1, B7-H4 and Foxp3$^+$ Tregs in gastric cancer and its clinical significance

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Abstract
Objective Immune escape plays an important role in tumor progression. In the present study, the expression of B7-H1, B7-H4 and Foxp3 involved in immune escape in gastric carcinoma was investigated and the corresponding clinical significance was evaluated.
Methods Immunohistochemistry was used to detect the expression of B7-H1, B7-H4 and Foxp3 in 100 gastric cancer specimens, and 30 paracarcinoma tissues were used as the control.
Results Both B7-H1 and B7-H4 showed high expression levels in gastric cancer tissues (65.0 and 71.0 %, respectively), and the expressions of B7-H1 and B7-H4 were positively correlated with the depth of tumor invasion, lymph node metastasis and American Joint Committee on Cancer (AJCC) stage ($P < 0.05$). The number of Foxp3$^+$ Tregs was much higher in gastric cancer tissues than control tissues, which was positively correlated with lymph node metastasis ($P < 0.05$). Similarly, a positive correlation between B7-H1 or B7-H4 expression and the number of Foxp3$^+$ Tregs was observed. The median overall survival rate of patients with high expression of B7-H1, B7-H4 and Foxp3 was significantly poorer than that of patients with low expression of these proteins ($P < 0.05$). Cox regression multivariate analysis confirmed that lymph node metastasis, AJCC stage, and B7-H1 and Foxp3 overexpression were independent prognostic factors.
Conclusion B7-H1, B7-H4 and Foxp3 were overexpressed in gastric cancer tissues. B7-H1 and Foxp3 are negative prognostic factors for patients with gastric cancer.

Keywords Gastric cancer · B7-H1 · B7-H4 · Foxp3 · Tregs · Costimulatory molecule

Introduction

Immune escape plays an important role in tumor progression [1]. Costimulatory molecules and the corresponding regulatory networks are involved in this progression. Recently, it has been reported that costimulatory molecules such as B7-H1 and B7-H4, two important inhibitory members of the B7 family, can suppress the immune response and induce immune escape by inhibiting the proliferation of T cells [2, 3]. B7-H1 has been reported to inhibit the proliferation of activated T cells and induce the apoptosis of T cells to form and maintain an immunosuppressive microenvironment [4]. It is overexpressed in patients with chronic diseases and virus infection, such as chronic hepatitis B (CHB) [5]. B7-H4, another important member of the B7 family, can induce immunosuppressive effects mainly through inhibiting the proliferation and activity of T cells, and down-regulating the secretion of immune cytokines such as IL-2 [6, 7]. Additionally, B7-H4

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may also be involved in the regulation of the human innate immune response [8]. Previous studies have detected the overexpression of B7-H1 and B7-H4 in many malignant tumors, including melanoma, breast, colorectal, esophageal, hepatocellular, pancreatic and gastric cancer, and even in some malignant hematological diseases such as leukemia and lymphoma [9–13]. The expression of B7-H1 and B7-H4 in the tumor microenvironment not only greatly suppresses the function of anti-tumor T cells and promotes growth and metastasis of tumors, but also potentially limits the efficacy of immune therapy [2]. In this study, we investigated the expression of B7-H1 and B7-H4 in gastric cancer tissues, and estimated their effects on tumor progression by analyzing the correlation between B7-H1 or B7-H4 expression and various clinicopathological characteristics. Meanwhile, we also evaluated the prognostic significance of these proteins in gastric cancer in order to provide more evidence for the therapy of gastric cancer.

Regulatory T cells (Tregs), a kind of negative regulatory T cell with the capability of immune suppression, are widely observed in human blood, tissues and organs. They play an important role in maintaining autoimmune stability, tumor immune tolerance and escape [14]. Forkhead/winged-helix transcription factor 3 (Foxp3), the specific molecular marker of Tregs located on chromosome Xp11.23, contains 11 exons and 10 introns [15, 16]. Recently, several reports have found that the number of Tregs was significantly increased in some tumor tissues [17, 18], and that the Tregs infiltrated into the tumor microenvironment could prevent the attack of natural killer (NK) cells and cytotoxic lymphocytes (CTLs) on tumor cells, by which they participate in immune inhibition-mediated tumor escape [19].

Gastric cancer in China has different environmental and genetic triggers from that in Western countries. In this investigation, the expression of Foxp3 in gastric cancer tissues was determined in order to analyze the correlation between Foxp3 expression and clinicopathological features, as well as the survival rate of Chinese patients. More importantly, in order to provide evidence for exploring the effects of B7-H1 and B7-H4 on Tregs, we evaluated the association between B7-H1 or B7-H4 and Foxp3 expression and explored the mechanism of immune escape induced by these proteins.

Patients and methods

Patients

The study included 100 gastric cancer patients treated in the Third Affiliated Hospital of Soochow University from 2005 to 2006. Gastric cancer samples were harvested from surgically resected specimens, and the paracarcinoma gastric mucosa tissues of 30 patients served as the negative control. All specimens were examined histologically according to the Japanese Classification of Gastric Carcinoma [20] by two pathologists independently. Disagreements were resolved by discussion. All patients were followed up for >5 years. Overall survival (OS) was measured from the date of surgery to the date of death.

The experimental protocols were approved by the appropriate institutional review committee and met the guidelines of the responsible governmental agency.

Immunohistochemistry (IHC)

Immunohistochemical staining was performed using the Elivsion^{TM} method. The following primary antibodies were used in this study for B7-H4 (USCN Life Science, USA), B7-H1 (clone 2H11, Novus, USA) and Foxp3 (Novus, USA). The second antibody and diaminobenzidine tetrahydrochloride (DAB) solution were provided by Maxim Company (Fuzhou, China).

All samples were fixed in formalin solution and embedded in paraffin. Sections (3–4 μm) were dewaxed in xylene, dehydrated in ethanol, and incubated in 3 % H_2O_2 for 15 min to destroy the activity of endogenous peroxidase. After incubation in 10 % normal bovine serum for 10 min, each slide was incubated with the primary antibodies at 4 °C overnight. Biotin-labeled mouse-rabbit immunoglobulin was chosen as the second antibody. The positive and negative controls were provided by the manufacturer.

Evaluation of immunostaining

Immunostaining was scored by two pathologists. The intensity (I) of staining was graded on a scale of 0–3+, with 0 representing no detectable staining and 3+ representing the strongest staining. Four strongest staining regions were randomly selected under a 40× field. In each of the four regions, the rate of positive cell staining (R) under a 400× field was calculated. R was defined as: 0, no staining; 1, ≤10 % tumor cells with staining; 2, 11–50 % tumor cells with staining; 3, 51–75 % tumor cells with staining; and 4, >75 % tumor cells with staining. Samples with scores <3 were considered as the negative and with scores ≥3 were considered as the positive. Histochemistry score = $I \times R$. The mean optical density of B7-H1 and B7-H4 was analyzed by Image-Pro Plus 6.0 software.

The distribution of Foxp3$^+$ Tregs was observed at low magnification first, and then five regions were selected randomly to calculate the rate of positive cell staining under a 400× field (negative ≤25 %, positive >25 %).

Statistical analysis

The correlations between the expressions of B7-H1, B7-H4 or Foxp3 and clinicopathological characteristics were analyzed by the χ^2 test. The correlations of the expression levels of B7-H1, B7-H4 and Foxp3 were analyzed by Spearman correlation coefficients. The influence of these proteins on survival was assessed by the Cox proportional hazards model using the backward-LR method. Survival curves were plotted using the Kaplan–Meier method and the statistical difference was analyzed using the log-rank test. For all statistical analyses, SPSS 17.0 software (SPSS, Chicago, IL, USA) was used, and a significant difference was considered at $P < 0.05$.

Results

Patients' characteristics

There were 61 males and 39 females in the postoperative patients with an age range of 30–87 years (median 66.4 years), of which 31 patients were well or moderately differentiated, and 69 patients were poorly differentiated. In addition, 68 patients had lymph node metastasis. According to the American Joint Committee on Cancer (AJCC) standard, all patients were staged from IB to IV in this study: there were 40 patients in stages I and II and 60 patients in stages III and IV. Other clinicopathological features are shown in Table 1. All of these patients received adjuvant chemotherapy (5-FU-based regimens, including cisplatin + 5-FU or FOLFOX4 for 4–6 cycles) after radical resection.

Expression of B7-H1 and B7-H4 and Foxp3$^+$ Tregs in gastric cancer and normal tissues

B7-H1- and B7H4-positive staining was detected in 65.0 and 71.0 % of gastric cancers, respectively, and the positive rates were significantly higher in gastric cancer tissues than paracarcinoma normal tissues. No B7-H1 or B7-H4 staining or only very weak staining was observed in the normal mucosa. Immunohistochemistry showed that the expression of B7-H1 was distributed mainly in the cytoplasm and partly in the nucleus (31.0 %) of gastric cancer cells. B7-H4 protein was mainly expressed in cytoplasm

Table 1 Correlation between B7-H1, B7-H4 or Foxp3 expression and clinicopathological features

Clinicopathological features	*n*	B7-H1		χ^2	*P* value	B7-H4		χ^2	*P* value	Foxp3		χ^2	*P* value
		+	−			+	−			+	−		
Gender													
Male	61	42	19	1.02	0.312	46	15	1.477	0.224	48	13	0.620	0.431
Female	39	23	16			25	14			28	11		
Age													
>60 years	65	47	18	3.490	0.062	49	16	1.734	0.188	56	9	10.487	0.001**
≤60 years	35	18	17			22	13			20	15		
Size													
≤5 cm	70	48	22	1.308	0.253	51	19	0.391	0.532	53	17	0.010	0.919
>5 cm	30	17	13			20	10			23	7		
Differentiation													
Poor	69	48	21	0.251	0.671	52	17	2.057	0.151	56	13	3.248	0.071
Well/moderate	31	17	14			19	12			20	11		
Invasion depth													
Muscle/serosa	67	47	20	2.366	0.095	53	14	6.477	0.011*	52	15	0.289	0.591
Mucosa	33	18	15			18	15			24	9		
Lymph node metastasis													
Yes	68	50	18	6.795	0.009**	54	14	7.303	0.007**	57	11	7.131	0.016*
No	32	15	17			17	15			19	13		
Staging													
III–IV	60	47	13	11.722	0.001**	49	11	6.610	0.004**	47	13	0.448	0.503
I–II	40	18	22			22	18			29	11		
Lymphatic or venous invasion													
Yes	42	23	19	2.606	0.090	27	15	1.073	0.265	30	12	0.454	0.477
No	58	42	16			44	14			46	12		

* $P < 0.05$, ** $P < 0.01$

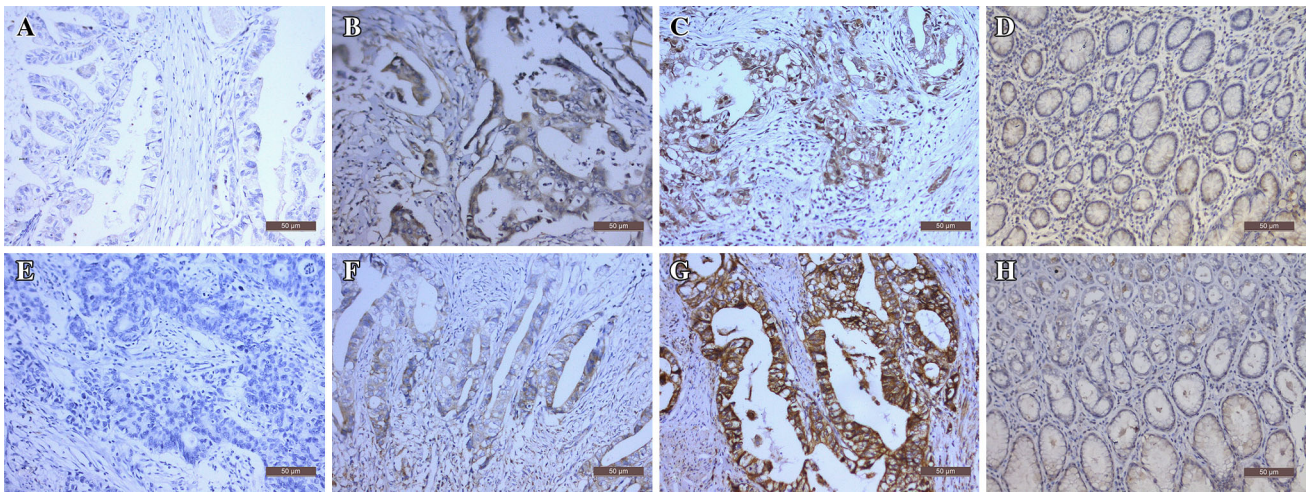

Fig. 1 B7-H1 and B7H4 immunohistochemistry in gastric cancer and normal tissues. B7-H1 and B7-H4 stained brown. **a** Negative expression of B7-H1 in gastric cancer tissues (0). **b** Weak positive expression of B7-H1 in gastric cancer tissues (+). **c** Strong positive expression of B7-H1 in gastric cancer tissues (+++). **d** Negative expression of B7-H1 in normal tissues. **e** Negative expression of B7-H4 in gastric cancer tissues (0). **f** Weak positive expression of B7-H4 in gastric cancer tissues (+). **g** Strong positive expression of B7-H4 in gastric cancer tissues (+++). **h** Negative expression of B7-H4 in normal tissues (magnification ×400)

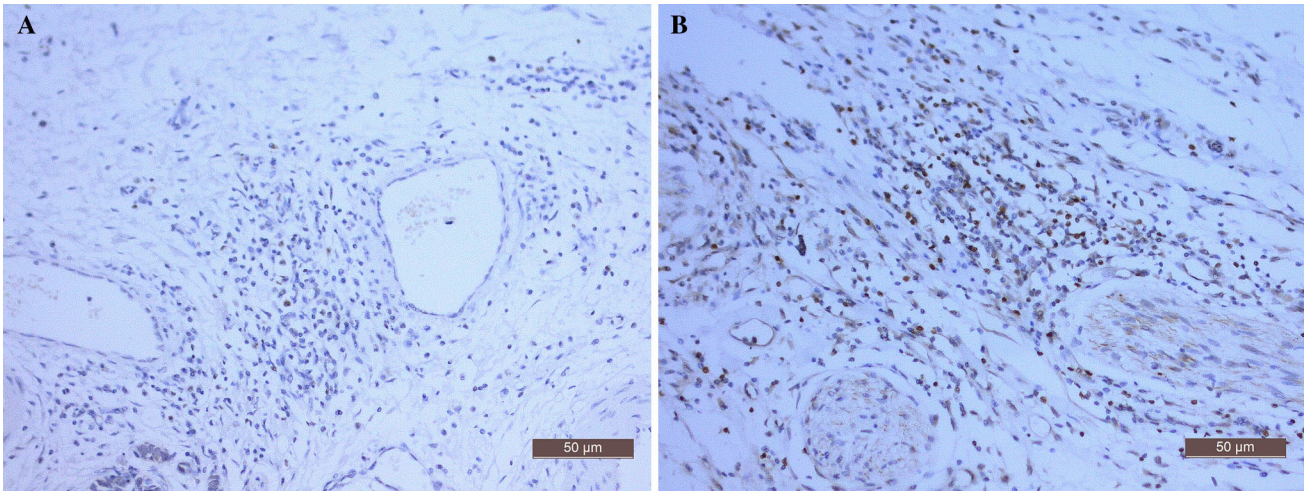

Fig. 2 Foxp3^{+} Tregs infiltrated in normal and gastric cancer tissues. **a** Foxp3-negative expression in normal tissues (≤25 %). **b** Foxp3-positive expression in gastric cancer (>25 %)

and membrane of gastric cancer cells, and rarely in the nucleus (Fig. 1).

The expression of Foxp3 was distributed mainly in the nucleus of T lymphocytes infiltrated in gastric cancer tissues, and the positive rate in gastric cancer tissues reached up to 76.0 %. However, little Foxp3 staining in the normal mucosa was observed (Fig. 2).

Correlation between B7-H1, B7-H4 or Foxp3 expression and clinicopathological features

The positive rates of B7-H1 and B7-H4 were significantly higher in tissues with lymph node metastasis and late AJCC than in tissues without lymph node metastasis or with early AJCC stage (B7-H1: lymph node^{+}, 73.5 %; lymph node^{-}, 46.9 %; $P = 0.009$. Stages III and IV, 78.3 %; stages I and II, 45.0 %; $P = 0.001$. B7-H4: lymph node^{+}, 90.0 %; lymph node^{-}, 53.1 %; $P = 0.007$. Stages III and IV, 81.7 %; stages I and II, 55.0 %; $P = 0.004$). In addition, the expression of B7-H4 was also positively associated with the depth of tumor invasion. The positive rate of B7-H4 was higher in tissues invaded into muscle or serosa than in tissues invaded into mucosa (79.1 vs. 54.5 %, $P = 0.011$). However, no significant difference was observed in gender, age, tumor size, differentiation, lymphatic or venous invasion ($P > 0.05$), as shown in Table 1.

Table 2 Correlation between B7-H1, B7-H4 and Foxp3 expression in gastric cancer tissues

Spearman correlation analysis ($n = 100$)	B7-H1	B7-H4	Foxp3$^+$ Tregs
B7-H1			
r	1.000	0.524	0.437
P	–	0.042*	0.013*
B7-H4			
r	0.524	1.000	0.456
P	0.042*	–	0.021*
Foxp3			
r	0.437	0.456	1.000
P	0.013*	0.021*	–

* $P < 0.05$

In addition, a positive correlation between the number of Foxp3$^+$ Tregs and lymph node metastasis was confirmed. Foxp3-positive staining was detected in 83.8 % of cases with lymph node metastasis, and was significantly lower in lymph node-negative specimens (59.4 %, $P = 0.016$). Moreover, Foxp3 revealed a higher expression level in patients aged >60 years than younger patients (86.2 vs. 57.1 %, $P = 0.001$). In contrast, no statistical association was seen between Foxp3 and other features such as gender, tumor size, differentiation, invasion depth or AJCC stage ($P > 0.05$), as shown in Table 1.

Correlation between B7-H1/B7-H4 expression and the number of Foxp3$^+$ Tregs in gastric cancer tissues

A positive correlation between B7-H1/B7-H4 expression (evaluated by mean optical density) and the number of Foxp3$^+$ Tregs in gastric cancer tissues was confirmed by Spearman correlation analysis. The correlation coefficients (r) were 0.437 ($P = 0.013$) and 0.456 ($P = 0.021$), respectively. In addition, the expression of B7-H1 was also positively correlated with B7-H4 ($r = 0.524$, $P = 0.042$), as shown in Table 2.

Survival analysis

All patients were followed up for more than 5 years. Cox regression univariate analysis showed that poor tumor differentiation, lymph node metastasis, late AJCC stage, and B7-H1, B7-H4 and Foxp3 overexpression were negative prognostic factors for OS ($P < 0.05$, Table 3). However, other factors such as age, gender, size and invasion depth of tumor had no effect on survival of patients ($P > 0.05$). Moreover, Cox regression multivariate analysis confirmed that lymph node metastasis, AJCC stage, and B7-H1 and Foxp3 expression were independent prognostic factors [hazard ratio (HR) = 1.69, 95 % confidence interval (CI) = 1.23–2.55, $P = 0.005$; HR = 3.18, 95 % CI = 2.18–4.03, $P = 0.011$; HR = 2.12, 95 % CI = 1.59–2.34, $P = 0.023$; HR = 1.65, 95 % CI = 1.12–3.58, $P = 0.036$, respectively, Table 3].

The Kaplan–Meier curves are shown in Fig. 3. The median OS of patients with B7-H1-positive expression was significantly poorer than B7-H1-negative cases (28.0 vs. 60.0 months, log-rank test, $P = 0.026$, Fig. 3a). The median OS of patients with B7-H4-positive expression was also significantly poorer than B7-H4-negative cases (36.0 vs. 60.3 months, log-rank test, $P = 0.048$, Fig. 3b). Meanwhile, the negative prognostic value of Foxp3$^+$ Tregs was revealed. Patients with Foxp3$^+$ Tregs-positive infiltration were confirmed to have poorer survival than the Foxp3$^+$ Tregs-negative group (36.0 vs. 48.0 months, log-rank test, $P = 0.023$, Fig. 3c).

Discussion

Gastric cancer is a common malignant tumor with high morbidity and mortality [21], especially in China [22]. Despite the availability of various treatment approaches including surgery, radiotherapy and chemotherapy, the 5-year survival rate of patients with gastric cancer is still poor. Gastric carcinogenesis is a complicated process involving many steps and aberrant genes such as the activation of oncogenes or the inactivation of tumor suppressor genes. Recently, a variety of studies have shown that the tumor microenvironment plays an important role in tumorigenesis and progression. The abnormality of gene expression or biological behavior of the cells in the tumor microenvironment will mediate tumor development and metastasis through the interaction with tumor cells. Immune escape has been confirmed as an important motivating factor in this process. As the only molecules that can transmit signals from antigen-presenting cells to T cells, costimulatory molecules in the B7 family are involved in the regulation of immune response, inflammation and malignant tumors [23].

The human B7-H1 gene, located in chromosome 9p24.2, encodes a type I transmembrane protein containing IgV- and IgC-like regions with 290 amino acids. Studies have reported that B7-H1 and B7-H4 can be detected in several types of carcinoma including thymus, breast, liver, lung, colon, bladder cancer and squamous cell carcinoma of the tongue [10, 24]. However, there is very weak expression of B7-H1 and B7-H4 in non-tumor tissues [24, 25]. In this study, both B7-H1 and B7-H4 were detected in gastric cancer tissues, and the positive staining rates were much higher in cancer tissues than in benign controls. B7-H4 is also expressed on the surface of tumor-associated

Table 3 Univariate and multivariate analysis of the clinicopathological and molecular features for overall survival

Factor	Univariate analysis		Multivariate analysis	
	HR (95.0 % CI)	*P* value	HR (95.0 % CI)	*P* value
Gender				
Male vs. female	1.65 (0.58–1.88)	0.084	–	–
Age				
>60 years vs. ≤60 years	0.85 (0.71–1.16)	0.476	–	–
Size				
≤5 cm vs. >5 cm	0.98 (0.44–1.55)	0.215	–	–
Differentiation				
Poor vs. well/moderate	2.28 (1.04–4.99)	0.039*	1.52 (0.67–3.46)	0.320
Invasion depth				
Muscle/serosa vs. mucosa	1.24 (0.56–2.75)	0.589	–	–
Lymph node metastasis				
Positive vs. negative	3.08 (2.23–4.18)	0.016*	1.69 (1.23–2.55)	0.005**
Staging				
Stage III–IV vs. stage I–II	2.43 (1.68–2.94)	0.008**	3.18 (2.18–4.03)	0.011*
B7-H1				
Positive vs. negative	1.65 (1.16–2.73)	0.038*	2.12 (1.59–2.34)	0.023*
B7-H4				
Positive vs. negative	1.41 (1.01–1.98)	0.049*	1.56 (0.91–2.45)	0.098
Foxp3				
Positive vs. negative	1.74 (1.04–2.36)	0.022*	1.65 (1.12–3.58)	0.036*

* $P < 0.05$, ** $P < 0.01$

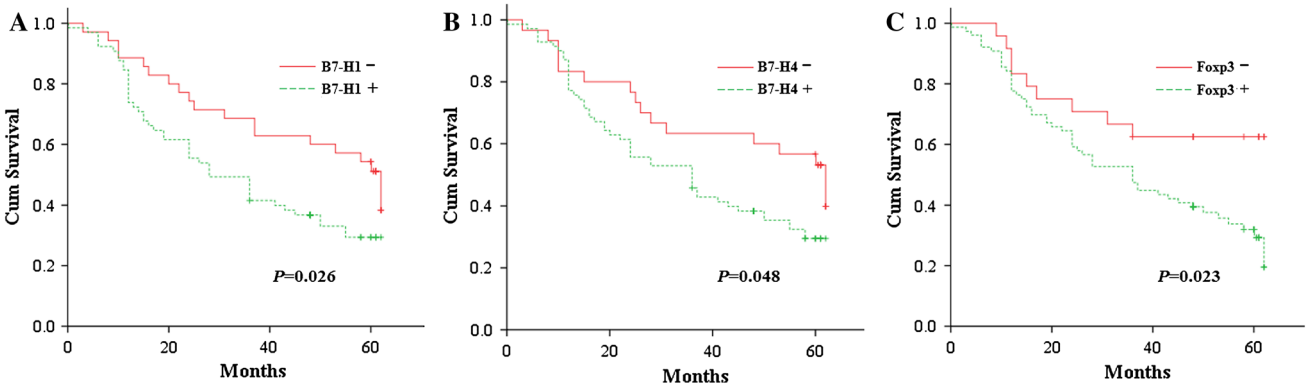

Fig. 3 Survival curves for patients with different expression levels of B7-H1, B7H4 and Foxp3. **a** OS of B7-H1-positive patients was significantly shorter than that of B7-H1-negative patients (28.0 vs. 60.0 month, $P = 0.026$). **b** OS of B7-H4-positive patients was significantly shorter than that of B7-H4-negative patients (36.0 vs. 60.3 month, $P = 0.048$). **c** OS of patients with Foxp3$^+$ Tregs-positive infiltration was significantly poorer than that of patients with Foxp3$^+$ Tregs-negative infiltration (36.0 vs. 48.0 months, $P = 0.023$)

macrophages and vascular endothelial cells of renal cell carcinoma (RCC) [26, 27]. However, no significant expression of B7-H4 in gastric stromal cells was reported.

According to previous reports, there is no statistically significant relationship between B7-H4 expression and clinicopathological characteristics such as differentiation, stage etc. in breast cancer [28], and no prognostic value of B7-H4 for ovarian cancer patients [29]. However, the expression of B7-H4 in RCC has been reported to be associated with adverse clinicopathological features, such as advanced tumor size, grade and stage [27]. RCC and prostate carcinoma patients with B7-H4-positive tumors usually have a high risk of recurrence and death [30]. In our study, the expression levels of B7-H1 and B7-H4 were significantly higher in samples with muscle or serosa invasion, lymph node metastasis and later stage. The expression of B7-H1 and B7-H4 was not associated with other clinicopathological features such as gender, age, tumor size and histological differentiation. Therefore, the expression of B7-H1 and B7-H4 seemed to be associated

with more aggressive gastric cancer. These results were consistent with the previous reports [12, 13]. We have also confirmed a significant prognostic value of B7-H1 and B7-H4 in gastric cancer by Cox regression analysis. The OS of the patients with B7-H1- or B7-H4-positive expression was significantly poorer than negative ones ($P < 0.05$), suggesting that in gastric cancer, B7-H1 and B7-H4 can promote tumor invasion and metastasis by regulating immune response and affecting the tumor microenvironment. Patients with B7-H1 or B7-H4 overexpression generally have a higher population of malignant phenotype and poorer survival due to the absence of normal immune surveillance. Thus, the detection of B7-H1 and B7-H4 expression in gastric cancer tissues is helpful for predicting the prognosis of patients and can be used for deciding treatment strategies. More powerful treatment approaches need to be considered for patients with higher expression of B7-H1 or B7-H4, and patients with higher B7-H1 or B7-H4 expression may benefit from B7-H1 and B7-H4 blockade. Soluble B7-H4 can be detected in the blood of cancer patients [31, 32]. Perhaps the serum level of B7-H4 has important diagnostic and prognostic value for gastric cancer, which needs further investigation and verification.

Expression of B7-H1 and B7-H4 in tumor cells may weaken the immunogenicity of tumors and influence the response of specific T cells. Transferring the B7-H1 gene into the mouse mastocyte tumor cell line P815 can enhance the growth of tumors [33]. Apoptosis of $CD8^+$ CTL is involved in B7-H1-induced immune escape [34] and can be restrained by anti-B7-H1 monoclonal antibody [33]. Curiel et al. [35] found that immunotherapy (dendritic cells + CTLs) combined with anti-B7-H1 monoclonal antibody can efficiently enhance tumor clearance rate by up-regulating the level of IFN-γ secreted by $CD8^+$ T cells in tumor infiltration regions. In an in vitro trial, B7-H4 inhibited the proliferation of $CD4^+$ and $CD8^+$ T cells, cytokine production, and generation of alloreactive CTLs by arresting the cell cycle [36–38]. In addition, B7-H4 expressed on the surface of antigen-presenting cells (APCs) also inhibits T-cell proliferation [37, 38]. B7-$H4^+$ tumor macrophages suppress tumor-associated antigen-specific T-cell immunity and the T-cell-stimulating capacity of macrophages can be restored by B7-H4 blockade.

Tregs, also known as suppressor T cells (Ts), are a type of immune suppressive cells and participate in tumor immune escape. Foxp3 is the most specific surface marker of Tregs, which was of great use for the determination and separation of Tregs [39]. A large number of chemokines secreted by tumor cells and macrophages in the tumor microenvironment, such as CCL22 and CCL20, increase the number of Tregs and recruit Tregs from thymus, bone marrow, lymph nodes and peripheral blood to tumor regions [14, 40]. The number of $Foxp3^+$ Tregs infiltrated in tumor tissues is significantly increased and negatively associated with the prognosis of patients [17, 18]. In the present study, a large number of $Foxp3^+$ Tregs infiltrated in gastric cancer was observed, especially in patients with lymph node metastasis ($P < 0.05$). The median OS of patients with high expression level of Foxp3 was significantly shorter than the low Foxp3 expression group, indicating that $Foxp3^+$ Tregs make an important contribution to the development of gastric cancer. As a specific marker of Tregs, Foxp3 can be also considered as a negative prognostic indicator for patients with gastric carcinoma. Interestingly, a significantly positive association between Foxp3 expression and patients' age was observed in this study. The expression level of Foxp3 in patients with age >60 years was significantly higher than that in younger patients (86.2 vs. 57.1 %, $P = 0.001$). Because Foxp3 is a negative regulatory factor of the immune system, we attribute this result to the decline of immune function in older patients.

Tregs in vivo have the ability to remove autoreactive T cells and maintain immune tolerance. The reduction of Tregs probably leads to autoimmune diseases, and the elevation of Tregs will result in immune disorders and carcinogenesis. Tregs induce immune escape through secreting IL-10 and transforming growth factor β (TGF-β), which in turn promote the proliferation and differentiation of Tregs [41, 42]. Glucocorticoid-induced tumor necrosis factor receptor (GITR) is another factor which mediates immune inhibition. The inhibition effect of Tregs on cytokine-induced killer (CIK)-induced anti-tumor activation will be significantly weakened by blocking GITR or TGF-β in patients with lung cancer [43]. A growing body of evidence suggests the important role of the CD39–CD73–adenosine pathway in tumor escape [44]. Functional CD39 expression on $Foxp3^+$ Tregs suppressed anti-tumor immunity mediated by NK cells in vitro and in vivo, and the inhibition of CD39 activity by polyoxometalate-1 significantly restrained the growth of tumors [45].

In the present study, the correlation between B7-H1 or B7-H4 expression and the number of $Foxp3^+$ Tregs in gastric cancer tissues were also analyzed. We concluded that a positive correlation existed between B7-H1, B7-H4 and Foxp3 expression. This result demonstrates that besides their capability to inhibit the proliferation of activated T cells, costimulatory molecules such as B7-H1 and B7-H4 can exert immunosuppressive effects, possibly by raising the number of suppressor T cells, which mediates tumor immune escape and promotes cancer development. Speculating from another viewpoint, down-regulation or inhibition of B7-H1 and B7-H4 expression can probably reduce or block the proliferation of Tregs, normalize the immune function, and thus induce an anti-tumor effect. However, the specific regulatory mechanism of B7-H1 and

B7-H4 on Foxp3$^+$ Tregs and the clinical significance of these molecules in the diagnosis and therapy of gastric cancer remain unclear, which requires further basic and clinical investigations to provide more evidence and ideas on the treatment of gastric cancer.

Conclusion

The expression levels of B7-H1 and B7-H4 in gastric cancer tissues were higher than those in normal mucosa, and are significantly associated with lymph node metastasis, AJCC stage and Foxp3 expression. The significantly positive correlation between B7-H1, B7-H4 and Foxp3 suggests that B7 molecules may induce immune inhibition by up-regulating Foxp3$^+$ Tregs, and synergistically participate in carcinogenesis, invasion and metastasis. These molecules, especially B7-H1 and Foxp3, can be considered as poor prognostic factors; meanwhile, they can also be considered as new targets for the therapy of gastric cancer.

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Conflict of interest The authors declare that they have no conflict of interest.

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