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journal homepage: www.elsevier.com/locate/yexmpCorrelation between infiltration of FOXP3⁺ regulatory T cells and expression of B7-H1 in the tumor tissues of gastric cancerJingying Hou¹, Zhong Yu¹, Rengyun Xiang, Chuqiang Li, Lin Wang, Shufen Chen, Qingyun Li, Mei Chen, Linyun Wang^{*}

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ABSTRACT

Background: Substantial evidence suggests that the expansion of regulatory T cells (T_{regs}) plays a pivotal role in immunological evasion of tumors. Recent studies have demonstrated that a majority of tumor cells overexpress B7-H1, and this overexpression is associated with poor disease prognosis. Although an increase of T_{regs} and B7-H1 has been revealed in several malignancies, their correlation in gastric cancer has not been studied.

Methods: Tumor sections from 111 gastric cancer patients were stained for FOXP3 and B7-H1 by immunohistochemistry. The expression levels of these two molecules were statistically associated with various factors involved in disease progression and prognosis. The correlation between their expression levels was analyzed.

Results: The infiltration of FOXP3⁺ Tregs and expression of B7-H1 were observed in gastric cancer tissues, and there was a highly significant correlation between these two molecules ($P < 0.01$). The expression of FOXP3⁺ Tregs and B7-H1 was associated with lymph node metastasis and the clinicopathological stage and prognosis of gastric cancer patients. The expression levels of these two determinants in patients with lymph node metastasis and an advanced clinicopathological stage were distinctly higher ($P < 0.05$). The patients with enhanced expression of FOXP3⁺ Tregs and B7-H1 exhibited a lower overall survival rate and a worse prognosis ($P < 0.05$). **Conclusions:** Increased expression of FOXP3⁺ Tregs and B7-H1 was observed in gastric cancer tissues; the two molecules were closely correlated with each other, suggesting that they might be used as new biomarkers to predict the disease progression and prognosis. Combinatorial immunotherapeutic approaches based on depleting the T_{regs} and blocking B7-H1 might improve therapeutic efficacy in gastric cancer.

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Introduction

Regulatory T cells (T_{regs}) are a subset of T lymphocytes that possess an immunoregulatory capacity which allows them to suppress the proliferation and cytokine secretion of effector T lymphocytes. Inappropriate production or dysfunction of T_{regs} could cause severe damage to the host immune system. A decrease of the population of T_{regs} or attenuation of their function might lead to autoimmune diseases (Wang and Zheng, 2013), whereas an increase in T_{regs} or overactivation of this cell type promotes the progression of cancer (Hossain et al., 2013). Upregulation of T_{regs} in cancer patients results in immunological evasion of tumor cells and disease progression (Jang, 2013; Pan et al., 2012; Pedroza-Gonzalez et al., 2013). The expansion of T_{regs} in the peripheral blood, the draining lymph nodes and the tumor tissues of gastric cancer patients has been reported to be associated with lymph node metastasis

and an advanced clinicopathological stage. There is a reduction of the T_{regs} population upon resection of the primary tumor, however, their number returns to the pre-resection level once the disease relapses.

B7-H1 (also known as PDL1) is a member of the B7 superfamily. It is expressed in several human and murine tissues. Previous studies have demonstrated that B7-H1 is abundant in tumor cell lines and tumor tissues. Overexpression of B7-H1 in carcinomas has been shown to inhibit T-cell activation and proliferation while inducing apoptosis in the effector T cells, leading to diminished immune responses and the impairment of protective immunity against cancer. B7-H1 downregulates anti-tumor immune responses via its crosslinking with the receptor PD-1 (Hatam et al., 2012; Malaspina et al., 2011; Wenjin et al., 2012). PD-1 belongs to the CD28/CTLA-4 receptor subfamily and contains a cytoplasmic tail harboring an ITIM (immunoreceptor tyrosine-based inhibitory motif) and an ITSM (immunoreceptor tyrosine-based switch motif), which exerts a negative role to mediate it. A costimulatory function of B7-H1 exists once it is uncoupled from PD-1 binding (Kline and Gajewski, 2010), implying that receptors other than PD-1 might be involved in the regulation of its function. Upregulation of B7-H1 has been described in several malignancies, and this finding is closely

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correlated with the clinicopathological status of cancer patients (Chen et al., 2013; Shi et al., 2013). The expression of B7-H1 in gastric cancer has been revealed to be associated with various vital prognostic factors (Matsunaga et al., 2011).

B7-H1 has been confirmed to play a critical role in T_{regs} development and function maintenance. T_{regs} and B7-H1 perform analogous functions in the progression of autoimmune diseases, infectious diseases and transplantation tolerance. A recent study shows that B7-H1 synergizes with TGF- β to convert $CD4^+CD25^-$ -naive T cells into $CD4^+CD25^+T_{\text{regs}}$ (Francisco et al., 2009). In the same study, the authors have found that B7-H1-coated beads are able to induce T_{regs} cells in the absence of exogenous TGF- β , indicating that B7-H1 signaling can facilitate the development of induced T_{reg} cells (iT_{regs}). The B7-H1/PD1 pathway has been linked to the proliferation of T_{regs} and blockade of the B7-H1/PD1 pathway causes the abrogation of the T_{regs} function (Krupnick et al., 2005). Although the infiltration of $FOXP3^+T_{\text{regs}}$ and B7-H1 expression have been recorded in several types of tumor tissues (Hua et al., 2012; Ghebeh et al., 2008), their relationship to gastric cancer has not been evaluated.

This study is the first attempt to explore the correlation between $FOXP3^+T_{\text{regs}}$ and B7-H1 in gastric cancer. We detected the expressions of these two molecules in the tumor tissues of gastric cancer and investigated their associations with diverse clinicopathological parameters and the overall survival of the patients. The relationship of $FOXP3^+T_{\text{reg}}$ cells with B7-H1 was analyzed.

Methods

Patients and sample collection

Gastric cancer specimens were collected from 111 patients undergoing surgical excision of the primary tumor at the Sun Yat-sen Memorial Hospital of Sun Yat-sen University between July 2007 and February 2011. All of the patients included in the study had completed the follow-up until September 2011. The clinicopathological characteristics of the study subjects are summarized in Table 1. None of the patients received radiotherapy, chemotherapy or other medical intervention before the study. The patients with autoimmune diseases or who were taking immunosuppressive drugs were excluded from this study. Normal gastric mucosal tissues were obtained from 20 patients with perforation caused by gastric ulcers. All the tissues were fixed in formalin and embedded in paraffin for immunohistochemical staining. This study was approved by the Ethics Committee of Sun Yat-sen University, and written informed consent was acquired from each individual prior to the study.

Table 1
Clinicopathological characteristics of 111 gastric cancer patients.

Sex	Male	75
	Female	36
Age	Minimum	18
	Maximum	96
Size of tumor	<5 cm	57
	≥ 5 cm	54
Depth of infiltration	Mucous membrane layer	13
	Muscular layer	14
	Serous layer	84
Pathological pattern	Well-differentiated adenocarcinoma	13
	Moderately differentiated adenocarcinoma	20
	Poorly differentiated adenocarcinoma	60
	Other types	18
Site of primary tumor	Gastric fundus	21
	Gastric body	25
	Gastric antrum	85
Lymph node metastasis	Negative	33
	Positive	78
Clinicopathological stage	I + II	40
	III + IV	71

Immunohistochemical staining of tumor tissues

The tumor tissues and normal tissues were fixed with formalin and embedded in paraffin. Immunohistochemical staining was conducted on the paraffin-embedded, 4-mm thick sections of the specimens. Each section was dewaxed in xylene and rehydrated by a graded ethanol solution followed by antigen retrieval by heating the tissue in boiling citrate buffer (10 mmol/L, pH 6.0) for 20 min. The sections were cooled, immersed in 0.3% hydrogen peroxide (Invitrogen) for 15 min to block the endogenous peroxidase activity and rinsed in phosphate-buffered saline (PBS) for 5 min. The samples were blocked with 10% goat serum (Invitrogen) at room temperature for 15 min and incubated with primary antibodies against $FOXP3$ (rabbit anti-human polyclonal antibody, Sigma, diluted 1:150) and B7-H1 (rabbit anti-human polyclonal antibody, Abcam, diluted 1:250) at 4 °C overnight. The sections were dehydrated, cleared and incubated in goat anti-mouse/rabbit secondary antibody (Invitrogen) for 20 min and then coped with streptavidin-conjugated horseradish peroxidase (Invitrogen) for 20 min. The color was developed using DAB (Invitrogen), and the sections were counter-stained with instant hematoxylin (Sigma).

Cell counting

The percentage of cells that stained positive for $FOXP3$ and B7-H1 was assessed by an anatomical pathologist. $FOXP3$ was detected in the nuclei of the tumor infiltrating lymphocytes (TILs), whereas B7-H1 was observable in the cytoplasm or on the membrane of the tumor cells or the TILs. For the counting of the $FOXP3^+T_{\text{regs}}$ and B7-H1 $^+$ TILs, five areas in the tumor nest with the highest density of tumor-infiltrating lymphocytes were selected at low magnification first, and then the amount of positive tumor-infiltrating lymphocytes was determined and recorded in at least five random high power fields (HPF 400 $\times$ magnification). The number of B7-H1-positive tumor cells and $FOXP3$ -positive TILs was determined per 1000 tumor cells in each section. The percentages of the cells that were stained positive were calculated based on the five random areas selected, and the values were averaged before the statistical analysis was conducted. The cut-off point for the overexpression of $FOXP3$ and B7-H1 in the lymphocytes was 5%, whereas it was 10% for the overexpression of B7-H1 in the tumor cells.

Statistical analysis

Pearson's Chi-square (χ^2) test or Fisher's exact test was used in the correlation between the parameters where appropriate. Kaplan–Meier curves and the log-rank test were performed for the survival analysis. The SPSS 13.0 statistical software was applied for all the analyses. A two-tailed P-value less than 0.05 was considered to be statistically significant.

Results

$FOXP3^+T_{\text{regs}}$ infiltrated tumor tissues and correlated with disease progression and prognosis of gastric cancer patients

Abnormal increases of T_{regs} in cancer patients and their negative effect on immunotherapy have been confirmed by several studies. In our study, we investigated the expression of $FOXP3$ in tumor-infiltrating lymphocytes in gastric cancer tissues and analyzed its association with various clinicopathological parameters and the overall survival of the patients. We observed the expression of $FOXP3^+T_{\text{regs}}$ in 69% (77/111) of all the samples, and the percentage of $FOXP3^+T_{\text{regs}}$ in the tumor tissues was $11.0 \pm 0.7\%$. No $FOXP3^+T_{\text{regs}}$ were found in the normal gastric mucosal tissues ($P < 0.001$, Fig. 1a, d). The infiltration of $FOXP3^+T_{\text{regs}}$ was correlated with lymph node metastasis and the clinicopathological stage of the tumor ($P = 0.002$, $P = 0.004$, respectively, Table 2, Figs. 4

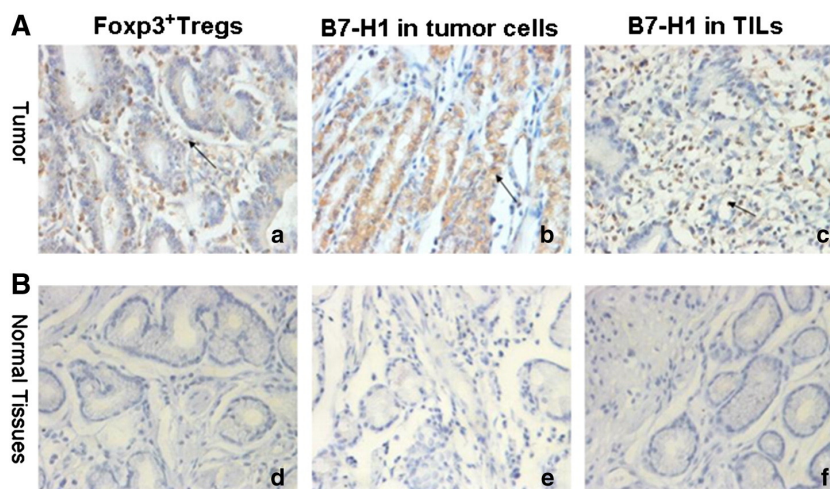


Fig. 1. Immunohistochemical staining for FOXP3 in lymphocytes and B7-H1 in tumor cells and TILs derived from gastric tissues. (A) Representative immunohistochemical staining of FOXP3 (nuclear, brown color) and B7-H1 (membrane or cytoplasmic, brown color) in tumor tissues. (B) Representative immunohistochemical staining of FOXP3 (nuclear, brown color) and B7-H1 (membrane or cytoplasmic, brown color) in normal tissues. The photomicrographs were at 400 $\times$ magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

and 5). FOXP3⁺ T_{regs} expression was discovered in those patients with lymph node metastasis and an advanced clinicopathological stage. The patients with a higher number of FOXP3⁺ T_{regs} showed a lower overall survival rate ($P = 0.015$, Figs. 2A, 7).

Upregulation of B7-H1 in gastric cancer tissue correlated with disease progression and prognosis of gastric cancer patients

Previous studies have revealed that overexpression of B7-H1 occurs in several types of tumors and correlates with the prognosis of the disease. In this study, we analyzed its expression in gastric cancer and assessed various clinicopathological parameters and the overall survival of the patients. We found the expression of B7-H1 in 63% (70/111) of the gastric cancer patients. The increased expression of B7-H1 was not restricted to the gastric cancer cells because 64% (71/111) of the patients showed B7-H1 overexpression in the TILs. The percentage of the B7-H1-positive tumor cells was $42.0 \pm 3.6\%$, and the percentage of the B7-H1-positive TILs was $17.2 \pm 1.6\%$. B7-H1 expression was not

observed in the glandular epithelium ($P < 0.001$) or in the lymphocytes ($P < 0.001$) derived from the normal gastric tissues (Fig. 1b, e, c, f). The analysis of the correlation between the B7-H1 expression in tumor cells and TILs and the clinicopathological parameters indicated that the upregulation of B7-H1 correlates significantly with lymph node metastasis (tumor cells $P < 0.001$, TILs $P = 0.003$) and an advanced clinicopathological stage (tumor cells $P < 0.001$, TILs $P = 0.007$, Table 3, Figs. 4 and 6). The survival analysis revealed that the overexpression of B7-H1 in tumor cells and TILs was closely associated with a lower overall survival rate (tumor cells $P = 0.033$, TILs $P = 0.021$, Figs. 2B, C, and 7).

Infiltration of FOXP3⁺ T_{regs} correlated with the upregulation of B7-H1 in gastric cancer

A correlation between FOXP3 and B7-H1 has been demonstrated in animal models. Studies investigating their relationship in cancer patients are relatively limited. In a recent study investigating the implication of T_{regs} and B7-H1 in breast cancer, the co-existence of FOXP3⁺ T_{regs}

Table 2

Correlation between expression of Foxp3⁺ Tregs and clinicopathological parameters of 111 gastric cancer patients.

		Foxp3		P
		+	–	
Sex	Male	52	23	0.991
	Female	25	11	
Age	<58	40	15	0.447
	≥ 58	37	19	
Size of tumor	<5 cm	38	19	0.526
	≥ 5 cm	39	15	
Depth of infiltration	Mucous membrane layer	8	5	0.396
	Muscular layer	8	6	
	Serous layer	61	23	
Pathological pattern	Well-differentiated adenocarcinoma	9	4	0.206
	Moderately differentiated adenocarcinoma	17	3	
	Poorly differentiated adenocarcinoma	37	23	
	Other types	14	4	
Site of primary tumor	Gastric fundus	17	4	0.435
	Gastric body	17	8	
	Gastric antrum	43	22	
Lymph node metastasis	Negative	16	17	0.002
	Positive	61	17	
Clinicopathological stage	I + II	21	19	0.004
	III + IV	56	15	

(+ and –) represented the numbers of the positive and negative patients respectively, P values in bold represented the data with significant differences.

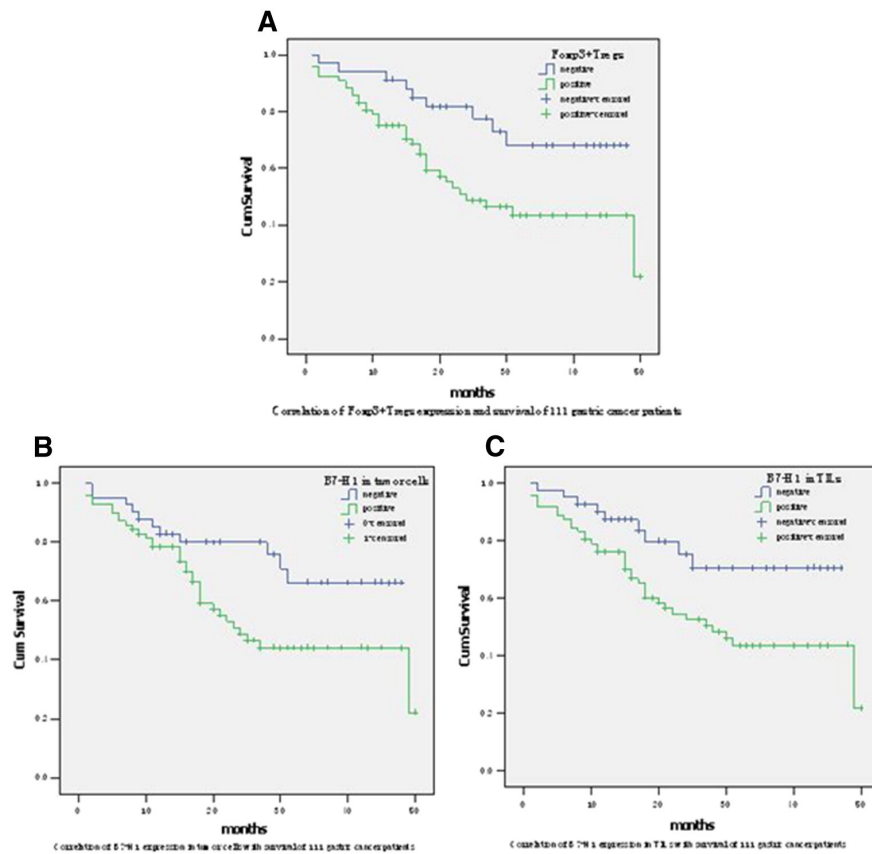


Fig. 2. Kaplan–Meier curves and log-rank test results for the overall survival of the patients with gastric cancer based on the expression of FOXP3⁺ T_{regs} and B7-H1 in tumor tissues. (A) The correlation of FOXP3⁺ T_{regs} expression with the overall survival of 111 gastric cancer patients. The log-rank test resulted in a P-value of 0.015. (B) The correlation of B7-H1 expression in tumor cells with the overall survival of 111 gastric cancer patients. The log-rank test resulted in a P-value of 0.033. (C) The correlation of the B7-H1 expression in TILs with the overall survival of 111 gastric cancer patients. The log-rank test resulted in a P-value of 0.021.

and B7-H1⁺ TILs was indicated. The results of this study revealed a significant correlation between FOXP3⁺ T_{regs} and B7-H1⁺ TILs in the tumor tissues. In this study, we extended the association between these two molecules in gastric cancer. Our results showed that the percentage of FOXP3⁺ T_{regs} correlated with the percentage of B7-

H1-positive tumor cells and TILs by Spearman's rank test (tumor cells $r = 0.479$, $P < 0.001$, TILs $r = 0.258$, $P = 0.006$). In our study, the tumor sections were classified into positive and negative groups according to the staining results. We discovered a significant correlation between the infiltration of FOXP3⁺ T_{regs} and the expression of B7-H1

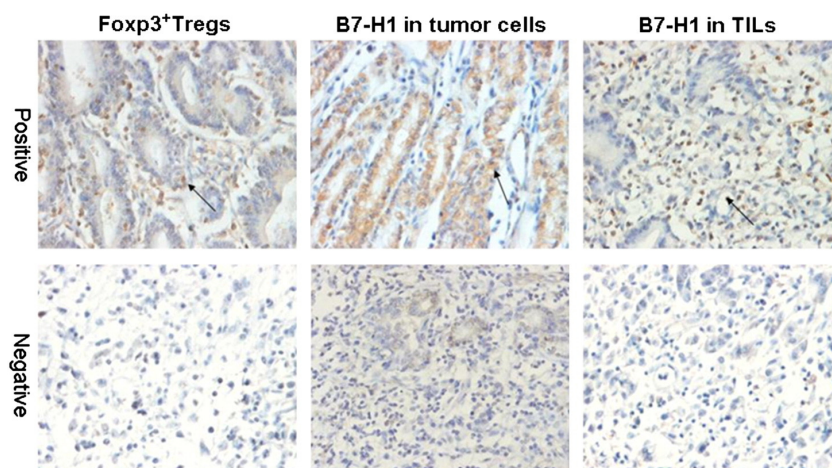


Fig. 3. Representative immunohistochemical staining of intratumoral FOXP3⁺ T_{regs} and B7-H1 in gastric cancer tissues. The expression was defined based on the intensity of the staining results. The photomicrographs were at 400× magnification.

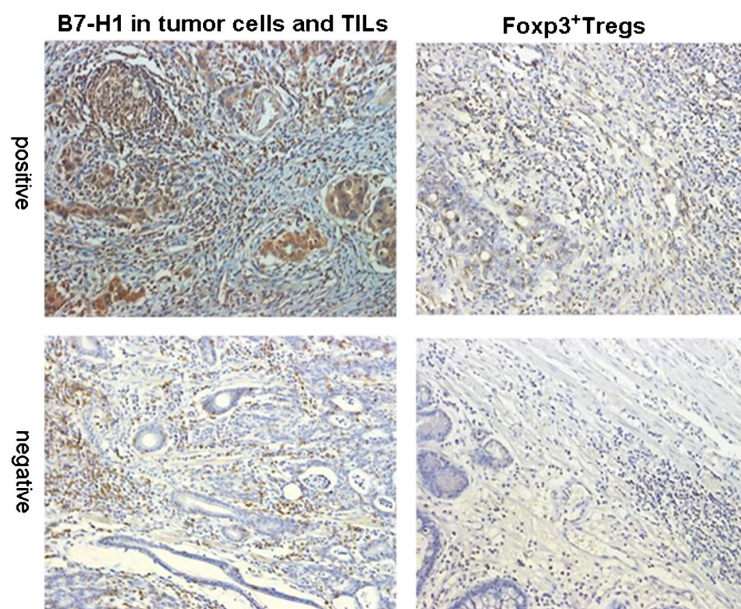


Fig. 4. Representative immunohistochemical staining of intratumoral FOXP3⁺ T_{regs} and B7-H1 in gastric cancer tissues derived from the patients with a different lymph node metastasis status. The expression was defined based on the intensity of the staining results. The photomicrographs were at 200 $\times$ magnification.

in the tumor cells and TILs (tumor cells $P < 0.001$, TILs $P = 0.004$, Fig. 3, Table 4).

Discussion

The maintenance of immune homeostasis involves intricate mechanisms mediated by various signaling molecules. During the past decade, abundant evidence has shown that T_{regs} exert essential roles in immunoregulation. Congenital deficiency or dysfunction of the T_{regs} population results in the disruption of self-tolerance. Conversely, the expansion of

T_{regs} in cancer patients could inhibit anti-tumor immune responses. The accumulation of T_{regs} in the tumor microenvironment might attribute to the overproduction of the suppressive cytokines or the migration of T_{regs} from other parts of the body via chemotaxis (Chen et al., 2003; Curiel et al., 2004; Walker et al., 2005; Yang et al., 2006). The specific mechanisms involved are unclarified and require further investigations.

Several studies have confirmed the infiltration of FOXP3⁺ T_{regs} in the tumor tissues in gastric cancer (Giuseppe et al., 2008; Mizukami et al., 2008). Intratumoral FOXP3-positive regulatory T cells are associated

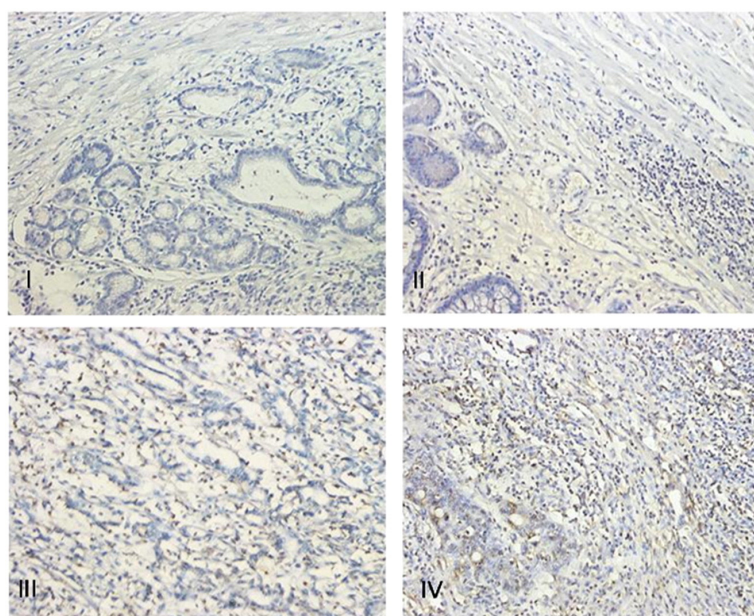


Fig. 5. Representative immunohistochemical staining of intratumoral FOXP3⁺ T_{regs} in gastric cancer tissues from the patients at different clinicopathological stages. The expression was defined based on the intensity of the staining results. The photomicrographs were at 200 $\times$ magnification.

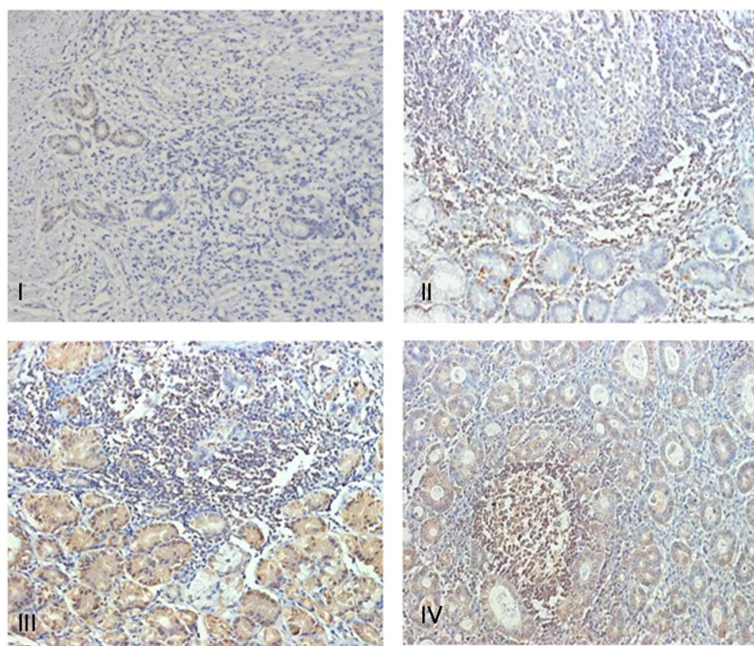


Fig. 6. Representative immunohistochemical staining of intratumoral B7-H1⁺ tumor cells and TILs in gastric cancer tissues derived from the patients at different clinicopathological stages. The expression was defined based on the intensity of the staining results. The photomicrographs were at 200 $\times$ magnification.

with adverse prognosis in radically resected gastric cancer, however, its correlation with various clinicopathological parameters and the prognosis of the disease is undetermined. In this paper, FOXP3 was used as a marker for T_{regs} because it has been commonly applied as a specific biomarker for studying T_{regs} in other malignancies. The expression of FOXP3⁺ T_{regs} was found in the tumor tissues of most gastric cancer patients. The infiltration of FOXP3⁺ T_{regs} in the tumor tissues was significantly associated with lymph node metastasis and clinicopathological stage. The discoveries indicated that the accumulation of FOXP3⁺ T_{regs} in the local tumor micro-environment might cause the escape of

tumor cells from immunosurveillance, eventually resulting in disease progression. The survival analysis displayed that the expression of the intratumoral FOXP3⁺ T_{regs} correlated with the prognosis of the patients. Our study shows that the FOXP3⁺ T_{regs} might represent a meaningful biomarker for predicting the progression and prognosis of gastric cancer.

Tumor cells attack the host immune system through elaborate strategies, including the downregulation of the cell surface major histocompatibility complex (MHC), the secretion of immunosuppressive cytokines, the suppression of costimulatory molecules and the expression of death

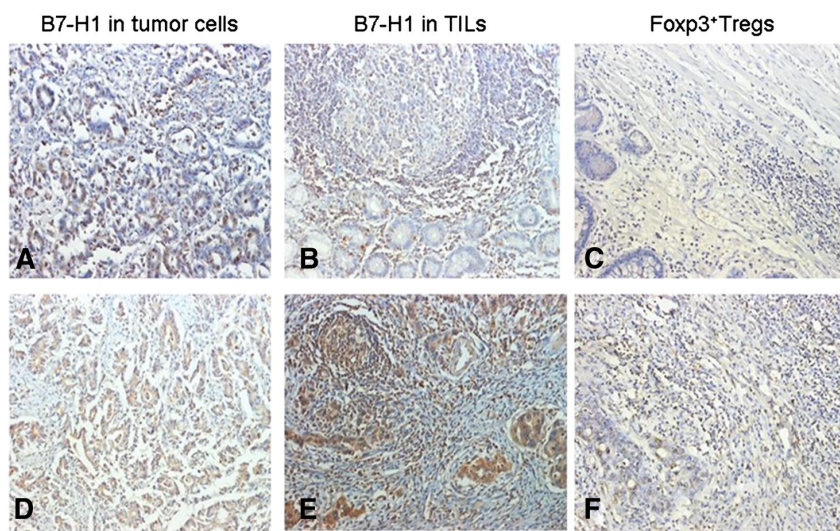


Fig. 7. Representative immunohistochemical staining of intratumoral B7-H1⁺ tumor cells and TILs in gastric cancer tissues derived from the patients of different overall survival rates. Panels A, B and C showed lower expression of these two markers, which was correlated with a higher overall survival rate. Panels D, E and F showed a higher expression of these two markers, which was correlated with a lower overall survival rate. The expression was defined based on the intensity of the staining results. The photomicrographs were at 200 $\times$ magnification.

Table 3

Correlation between expression of B7-H1 and clinicopathological parameters of 111 gastric cancer patients.

		B7-H1 in tumor cells			B7-H1 ⁺ TILs		
		+	–	P	+	–	P
Sex	Male	47	28	0.901	48	27	0.991
	Female	23	13		23	13	
Age	<58	33	22	0.508	36	19	0.746
	≥58	37	19		35	21	
Size of tumor	<5 cm	33	24	0.246	34	23	0.331
	≥5 cm	37	17		37	17	
Depth of infiltration	Mucous membrane layer	5	8	0.110	7	6	0.718
	Muscular layer	8	6		9	5	
	Serous layer	57	27		55	29	
Pathological pattern	Well-differentiated adenocarcinoma	6	7	0.070	8	5	0.370
	Moderately differentiated adenocarcinoma	16	4		16	4	
	Poorly differentiated adenocarcinoma	40	20		35	25	
	Other types	8	10		12	6	
Site of primary tumor	Gastric fundus	16	5	0.238	15	6	0.160
	Gastric body	17	8		12	13	
	Gastric antrum	37	28		44	21	
Lymph node metastasis	Negative	11	22	0.000	13	20	0.000
	Positive	59	19		58	20	
Clinicopathological stage	I + II	18	22	0.003	19	21	0.007
	III + IV	52	19		52	19	

(+ and –) represented the numbers of the positive and negative patients respectively, P values in bold represented the data with significant differences (P < 0.05).

ligands or negative ligands (Bhatia and Kumar, 2014; Rubin, 2009). Recently, B7-H1 has been identified as an immunoglobulin (Ig)-like inhibitory molecule that is detectable in a variety of cancers. B7-H1 interacts with its counter-receptor PD-1 and this subsequently leads to anti-tumor immune responses by provoking the apoptosis, anergy and unresponsiveness of the T cells (D'Addio et al., 2011; Fife et al., 2011; Francisco et al., 2009; Goldberg et al., 2007; Pulko et al., 2011; Tsushima et al., 2007). The upregulation of B7-H1 in the tumor cells and the negative immunoregulatory function it exerts in the local tumor microenvironment imply that the blockade of B7-H1 signaling could be a viable method in anti-tumor immunotherapy (Flies and Chen, 2007; Wang and Chen, 2011).

The expression of B7-H1 on several malignancies and its correlation with certain prognostic factors have been verified by a series of previous studies. In this study, we found an upregulation of B7-H1 in the tumor cells and tumor-infiltrating lymphocytes in gastric cancer tissues. Enhanced B7-H1 expression in tumor cells might attenuate their immunogenicity and impede effective host immune responses in this condition. B7-H1 expression was obviously correlated with lymph node metastasis and the clinicopathological stage. In addition, the B7-H1 positive group presented a lower overall survival rate. These results implied that B7-H1 might be involved in tumor metastasis and the prognosis of gastric cancer.

T_{regs} and the B7-H1/PD1 pathway are pivotal to the maintenance of peripheral immune tolerance. A novel role of B7-H1 in sustaining the function of iT_{regs} has been reported in other studies. B7-H1 modulates the signaling molecules that are critical for the conversion of naive T cells to T_{reg} cells. It triggers the reduction of phospho-Akt, mTOR, S6, and ERK2 while increasing the signaling from PTEN (Burton et al.,

2010; Zhao et al., 2007). B7-H1 promotes FOXP3 expression and intensifies its suppressive function (Jin et al., 2011). One previous report has documented that the co-existence of FOXP3⁺ TILs and B7-H1⁺ TILs in breast cancer tissues is significantly correlated with important prognostic factors of the patients (Krupnick et al., 2005). In this study, we found that the expression of these two molecules was associated with the clinicopathological factors and overall survival of the patients. An increase in the FOXP3⁺ T_{regs} was positively correlated with B7-H1 overexpression in tumor cells and TILs.

The most important findings of this study are the congregation of Foxp3⁺ T_{regs} and B7-H1 in the tumor microenvironment of gastric cancer and their close correlation. Because Foxp3⁺ T_{regs} and B7-H1 could counteract anti-tumor immune responses, a combined strategy based on the depletion of T_{regs} and the inhibition of B7-H1 should be developed to enhance therapeutic efficacy against gastric cancer. The potential benefits for this modality have been revealed in a recent animal renal cell carcinoma study, in which mice showed complete tumor regression and long-lasting protective immunity when subjected to this treatment (Webster et al., 2007). The clinical trials aimed at blocking the B7-H1/PD-1 pathway have manifested that B7-H1 inhibitors are safe and well-tolerated and that they have few autoimmune side effects (Ascierto et al., 2010; Brahmer et al., 2010). Despite these promising results, the specific implication of B7-H1 inhibitors on the T_{regs} function has not been reported and should be further explored in future studies of gastric cancer.

Conclusions

We demonstrated that the infiltration of FOXP3⁺ T_{regs} and expression of B7-H1 impinged on the disease progression and prognosis of gastric cancer patients. There was a significant correlation between these two molecules in the local tumor tissues. Our study provides a theoretical basis for targeting FOXP3⁺ T_{regs} and B7-H1 in the treatment of gastric cancer. Future investigations of these immunosuppressive molecules could be conducive to anti-tumor immunotherapy for gastric cancer.

Abbreviations

T _{regs}	regulatory T cells
ITIM	immunoreceptor tyrosine-based inhibitory motif
ITSM	immunoreceptor tyrosine-based switch motif
TIL	tumor-infiltrating lymphocytes

Table 4Correlation between infiltration of Foxp3⁺ Tregs and expression of B7-H1 in 111 gastric cancer patients.

		Foxp3 ⁺ Tregs		r	P
		+	–		
B7-H1 ⁺ tumor cells	+	59	11	0.389	0.000
	–	18	23		
B7-H1 ⁺ TILs	+	56	15	0.265	0.004
	–	21	19		

(+ and –) represented the numbers of the positive and negative patients, P values in bold represented the data with significant differences.

PDL1 (B7-H1) ligand of programmed death-1
PD1 programmed death-1

Competing interests

The authors declare that there are no conflicts of interest.

Authors' contributions

Jingying Hou and Zhongyu conducted the experiments, participated in the statistical analysis and drafted the manuscript. Rengyun Xiang, Qingyun Li and Shufen Chen participated in the sample collection and patient follow-up. Chuqiang Li, Mei Chen and Lin Wang provided technical assistance in the immunohistochemistry experiments. Linyun Wang conceived the study and participated in the study design. All the authors read and approved the final manuscript.

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