


ORIGINAL ARTICLE

Overexpression of B7H5/CD28H is associated with worse survival in human gastric cancer

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Abstract

Gastric cancer (GC) is a common malignancy with low 5-year overall survival (OS). Recently, immune therapy has been used to treat cancer. B7H5 and CD28H are novel immune checkpoint molecules. However, the prognostic value of B7H5/CD28H expression in patients with GC remains unclear. In this study, seventy-one patients diagnosed with GC were included in this study. Patients' GC tissues and matched adjacent tissue constructed a tissue microarray. The expression levels of B7H5 and CD28H were examined using immunohistochemistry. Correlations between the expression of B7H5 and CD28H and the clinical data were evaluated. We found that the expression of B7H5 and CD28H (both $P = .001$) were higher in GC tumour tissues than in adjacent noncancerous tissues. B7H5/CD28H expression acted as an independent predictive factor in the OS of patients with GC. High expression of B7H5 and CD28H predicted poor outcome. Patients in the B7H5+CD28H+ group had a lower 5-year OS compared with patients in the B7H5-CD28- group (4.5% vs 55.6%, $P = .001$). A significant difference was found in the 5-year OS between patients in the B7H5+CD28H- and B7H5+CD28H+ groups (33.5% vs 4.5%, $P = .006$). However, there was no correlation between B7H5 and CD28H expression ($P = .844$). Therefore, B7H5 and CD28H expression are up-regulated in GC and are independent prognostic factors for overall survival in patients with GC. Although there was no correlation between B7H5 and CD28H expression, high expression of B7H5 and CD28H predicts poor prognosis, especially when both are highly expressed.

KEYWORDS

B7 family checkpoints, B7H5, CD28H, gastric cancer, immunotherapy

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1 | INTRODUCTION

Gastric cancer (GC) is a common malignancy and has been estimated to account for one-third of cancer deaths.¹ Surgery is the optimal treatment for patients with GC, which provides the best chance of long-term survival. However, the early stage of the disease is often asymptomatic, and GC is frequently diagnosed in the later stages, when it is characterized by invasion or metastasis. The therapeutic options of neoadjuvant therapy, targeted drugs and immunotherapy have opened a new field of cancer treatment in recent years; however, the 5-year overall survival (OS) rate of GC is unsatisfactory.² While cancers suppress the immune response by establishing a microenvironment, the immune cells decrease cancer immunogenicity in the process of tumorigenesis and cancer progression.³ Therefore, the interaction between the microenvironment and the immune system is a new direction in GC research.⁴ However, patients with GC have not benefited from immunotherapy.

Immune checkpoint therapy is a novel treatment that targets regulatory pathways in T cells to enhance antitumour immune responses. The B7/CD28 family of ligands and receptors play an important role in cancer pathogenesis. Ten members are included in the B7/CD28 family, including B7-1/CD28, B7-2/cytotoxic T lymphocyte antigen 4 (CTLA-4), ICOS-L/ICOS and programmed cell death 1 (PD-1)/PD-1 ligand (PD-L1).⁵ PD-L1/PD-1 has become a hot area of research in recently years.^{6,7} The B7/CD28 family regulates the proliferation and function of T cells as antigen-presenting cells (APCs). In addition, some tumours can evade immune elimination, because of overexpression of PD-L1/PD-1.^{8,9} Inhibitors of PD-L1/PD-1 have been approved by Food and Drug Administration (FDA) as second-line treatments for lung cancer, because of their therapeutic benefit in clinical trials.

The B7H5/CD28H pathway is a novel receptor-ligand interaction in the B7/CD28 family that can reduce the expression of interleukin-5 (IL-5), IL-13, IL-10 and tumour necrosis factor gamma (TNF- γ) and suppress the activity of CD4+ and CD8+ T cells.¹⁰ Some studies showed low expression of B7H5 in normal tissue,¹¹ while B7H5 is overexpressed in some cancers.^{12,13} However, the prognostic value of B7H5/CD28H in patients with GC is controversial. In the present study, we aimed to evaluate the expression levels of B7H5 and CD28H in patients with GC.

2 | MATERIALS AND METHODS

2.1 | Tissue microarray (TMA) construction and immunohistochemistry (IHC) analysis

Seventy-one formalin-fixed, paraffin-embedded (FFPE) GC tissues and corresponding adjacent noncancerous tissues were collected from the Department of Abdominal Surgery, Zhejiang Cancer Hospital. All FFPE GC tissues were screened by two pathologists independently to confirm the diagnosis of GC. The most representative tumour and noncancerous tissues were selected to construct the TMA slide. Seventy-one paired GC tissues and matched noncancerous tissues were included in the TMA.

The IHC technique has been described in detail previously.¹⁴ TMA sections were deparaffinized, and hydrogen peroxide (3%) was applied to repair the antigen. Nonspecific staining was blocked using 10% goat serum at room temperature for 30 minutes. Immunostaining of histological sections was performed using monoclonal antibodies against B7H5 and CD28H (Gifts from Professor Yuwen Zhu, University of Colorado Anschutz Medical Campus) overnight at 4°C followed by a 30-minutes incubation with goat anti-rabbit IgG (H&L) Biotin secondary antibody (dilution 1:1,000; cat. no AB97049; BioVision, Inc)/goat anti-mouse IgG (H&L) Biotin secondary antibody (dilution 1:500; cat. no AB97049; BioVision, Inc) and visualization with 3,3'-diaminobenzidine (DAB) for 3 minutes. Harris haematoxylin was used to stain the nuclei. The sections were left to dehydrate and were then sealed with neutral gel. Categorization of the scanned images was performed using density quant software in the Quant Center (3DHitech, Ltd).

TABLE 1 Correlation between B7H5 expression and clinicopathological characteristics

Variables	B7H5 expression		Total	χ^2	P-value
	High	Low			
Age (y)					
≤66	26	9	35	0.039	.844
>66	16	20	36		
T stage					
T1/T2	4	6	10	4.141	.042 ^a
T3/T4	47	14	61		
TNM stage					
I/II	25	7	32	0.709	.400
III/IV	27	12	39		
N stage					
N0	17	2	19	3.488	.062
N1/N2/N3	35	17	52		
M stage					
M0	49	18	67	0.000	1.000
M1	3	1	4		
P53					
Negative	36	12	48	0.010	.922
Positive	17	6	23		
Sex					
Female	14	4	18	0.038	.845
Male	38	15	53		
Ki67					
Negative	18	14	32	8.579	.003 ^a
Positive	34	5	39		
Grade					
I/II	20	3	28	3.266	.071
III/IV	32	16	53		

^aStatistically significant ($P < .05$).

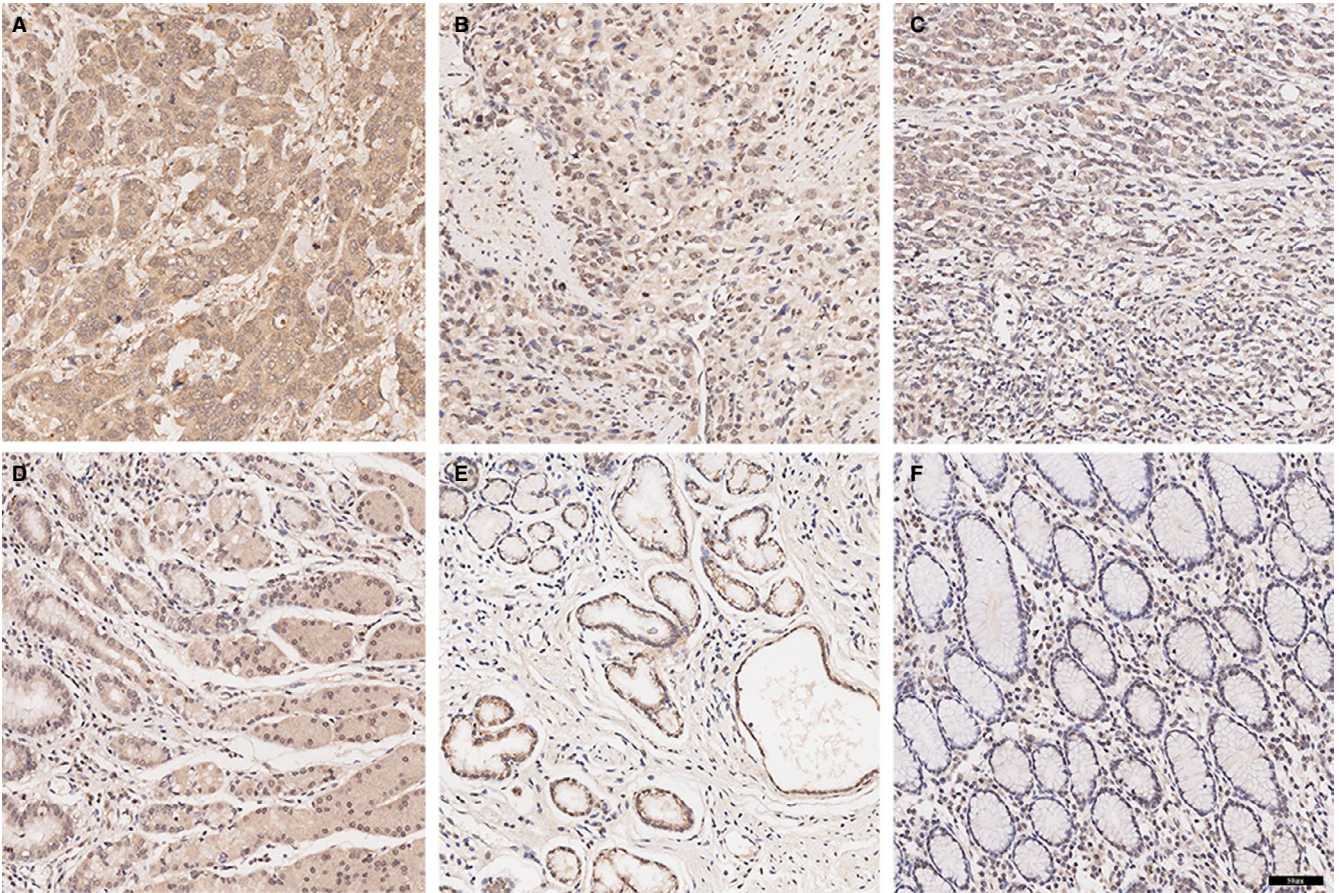


FIGURE 1 Expression of B7H5 in gastric carcinoma and noncancerous tissues. A-C, High/middle/low expression of B7H5 in gastric carcinoma. D-F, High/middle/low expression of B7H5 in noncancerous tissues

Cytoplasmic expression was assessed using the H-score system. The formula for the H-score was as follows: $H\text{-score} = \sum (IS \times AP)$, where IS represents the staining intensity and AP represents the percentage of positively stained tumour cells, producing a cytoplasmic score ranging between 0 and 12. An IS between 0 and 3 was assigned for the intensity of tumour cell staining (0, no staining; 1, weak staining; 2, intermediate staining; 3, strong staining). AP depended on the percentage of positive-stained cells as follows: 0 (0%), 1 (1%-25%), 2 (26%-50%), 3 (51%-75%) and 4 (75%-100%). The score was assigned using the estimated proportion of positively stained tumour cells. To assess the average degree of staining within a tumour sample, multiple regions were analysed, and at least 100 tumour cells were assessed. Two assessors who were blinded to the clinical outcomes performed the scoring independently.

2.2 | Flow cytometry

Ten fresh GC tissues and their corresponding adjacent noncancerous tissues were collected from the Department of Gastrointestinal Surgery, The First Affiliated Hospital of Zhejiang Chinese Medical University. Single-cell suspensions from the tissues were stained

for extracellular surface antigens using the anti-CD28H monoclonal antibodies, followed by incubation with goat anti-mouse IgG Biotin secondary antibody, FITC anti-human CD3 antibody and 7-AAD Viability staining solution. Finally, viable single cells were analysed.

2.3 | Post-operative evaluation and follow-up

Post-operative chemotherapy was performed according to each patient's individual pathological diagnosis. If an infiltrating margin was found, adjuvant irradiation was necessary. The patients were examined regularly every 3 months during the first year and every 6 months thereafter.

2.4 | Statistical analysis

Statistical analysis was performed using IBM SPSS statistics for Mac, version 23.0 (IBM Corp). Survival curves were estimated using the Kaplan-Meier method, and multivariate and univariate analyses were performed using a Cox stepwise proportional hazard model. A P -value $< .05$ was considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

Among the 71 patients with GC, 53 (74.65%) were male and 18 (23.35%) were female, with a median age of 67 (range 34-83) years. At the end of follow-up, 47 (66.20%) patients had died and 24 (33.80%) patients were alive. According to the depth of tumour invasion, 4 (5.63%) were at T1, 6 (8.45%) were at T2, 47 (66.20%) were at T3, and 14 (19.72%) were at T4. For lymph node metastasis, 19 (26.76%) were N0, 15 (21.13%) were N1, 21 (29.58%) were N2, and 16 (22.54%) were N3. For distant metastasis, 68 (95.77%) were M0 and 3 (4.23%) were M1. According to the 8th AJCC staging system, 7 (9.86%) were at stage I, 25 (35.21%) were stage at II, 35 (47.95%) were at stage III, and 4 (5.64%) were at stage IV (Table 1).

3.2 | B7H5 expression in cytoplasm in GC and noncancerous tissues

B7H5 protein levels in GC tissues are currently unknown. As shown in Figure 1, B7H5 was expressed in the cytoplasm in the noncancerous and GC tissues. However, the staining intensity of B7H5 in the cytoplasm in GC tissues was obviously stronger than that in noncancerous tissues (Figure 2A). The median H-score of B7H5 was 8 (range: 1-12). The median score was used to determine the cut-off value of low- or

high-level B7H5 expression. An H-score <8.0 was defined as low B7H5 expression, and an H-score \geq 8.0 was defined as high B7H5 expression. Nineteen (26.76%) patients showed low B7H5 expression, while 52 (73.24%) showed high B7H5 expression in GC tissues. However, 32 (45.07%) showed low B7H5 expression, and 39 (54.93%) patients showed high B7H5 expression in noncancerous tissues ($P = .001$).

3.3 | B7H5 expression in the nucleus in GC and noncancerous tissues

B7H5 was expressed in the nucleus in the noncancerous tissues and GC tissues. However, the staining intensity of B7H5 in the nuclei in GC tissues was obviously stronger than that in noncancerous tissues (Figure 2B). Forty-four (61.97%) patients showed low B7H5 expression, while 27 (38.03%) showed high B7H5 expression in GC tissues. However, 67 (94.37%) showed low B7H5 expression, and 4 (5.63%) showed high B7H5 expression in noncancerous tissues ($P < .001$).

3.4 | CD28H⁺ T cells exist in GC and noncancerous tissues

CD28H protein levels in GC tissues are currently unknown. As shown in Figure 3, the CD28H protein was expressed in both GC and noncancerous tissues. In the GC tissues and noncancerous tissues, CD28H was expressed in immune cells. However, the staining

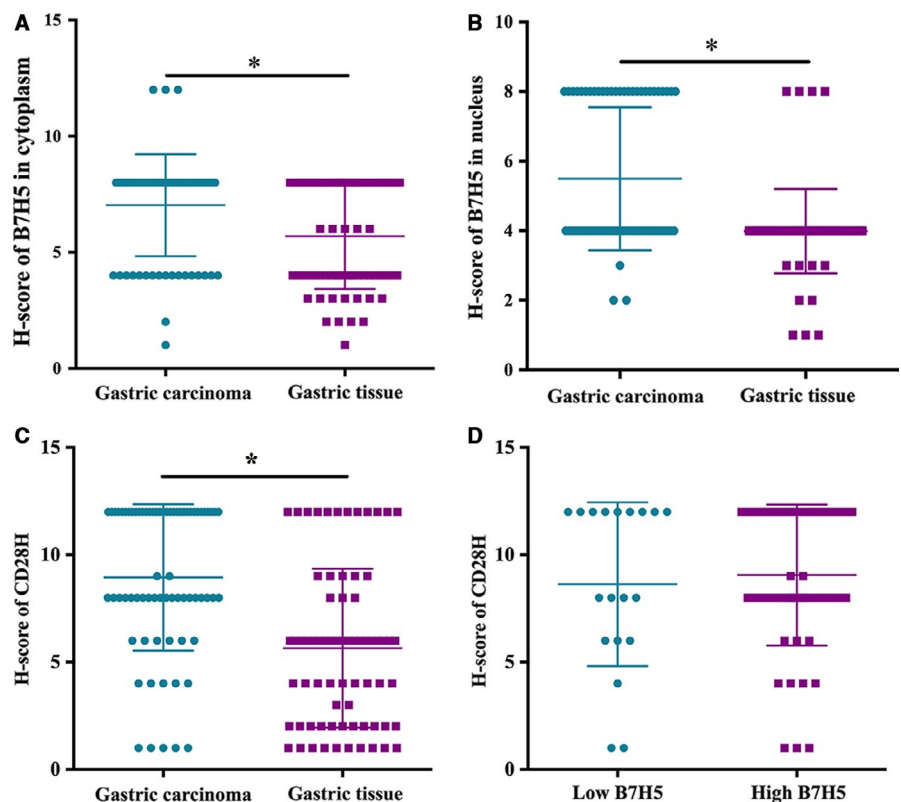


FIGURE 2 B7H5 protein was expressed in both gastric carcinoma and noncancerous tissues. A, B7H5 expression was higher in cytoplasm in GC tissues than in gastric tissue ($P = .001$); B, B7H5 expression was higher in the nucleus in GC tissues than in gastric tissue ($P < .001$); C, CD28h expression was higher in immune cells in GC tissues than in gastric tissue ($P = .001$); D, There was no correlation between B7H5 and CD28H expression ($P = .844$)

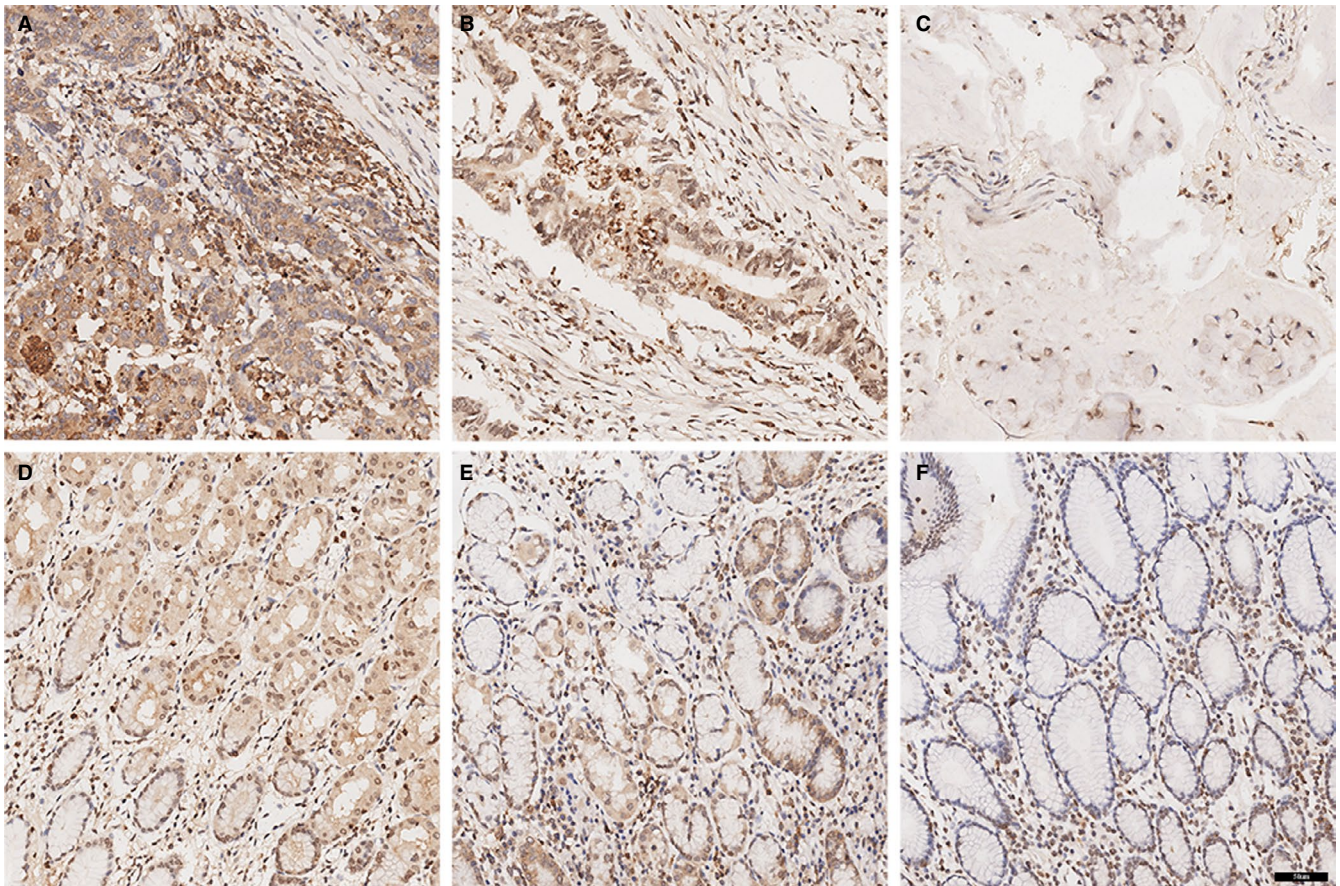


FIGURE 3 Expression of CD28H in gastric carcinoma and noncancerous tissues. A-C, High/middle/low expression of CD28H in gastric carcinoma. D-F, High/middle/low expression of CD28H in noncancerous tissues

intensity of CD28H in GC tissues was obviously stronger than that in noncancerous tissues. The median of H-score of CD28H was 8 (range: 1-12). The median score was used to determine the cut-off value of low or high CD28H expression. An H-score ≤ 8 was defined as low CD28H expression, and an H-score >8 was defined as high CD28H expression. Thirty-six (50.70%) patients showed low CD28H expression, while 35 (49.30%) showed high CD28H expression in GC tissues. However, 17 (23.94%) showed low CD28H expression, while 54 (76.06%) showed high CD28H expression in noncancerous tissues ($P = .001$). In addition, we analysed CD28H expression on CD3⁺T cells in GC tissues and gastric tissues using flow cytometry. As shown in Figure 4, the level of CD3⁺T cells in GC tissues was higher than that in gastric tissues ($P < .01$), while the level of CD3⁺CD28H⁺T cells was higher in GC than in gastric tissues ($P < .01$).

3.5 | B7H5 and CD28H expression in GC and clinicopathological variables

The association between B7H5 and CD28H expression and clinicopathological parameters in GC was investigated using the chi-square test. As listed in Table 1, a significant correlation was found between the depth of invasion (T) ($P = .042$) and Ki67 expression ($P = .003$),

while B7H5 expression in the cytoplasm was not significantly associated with age, sex, lymph node metastasis (N), distant metastasis (M), TNM stage, p53 expression or pathological grade. However, as shown in Tables 2 and 3, B7H5 expression in the nucleus and CD28H expression in immune cells was not significantly associated with age, sex, T stage, N stage, M stage, TNM stage, Ki 67 stage, p53 expression or pathological grade.

3.6 | Prognostic value of B7H5 and CD28H expression in patients with GC

As shown in Figure 5, the prognostic value of B7H5 expression in GC was investigated. Although there was no difference in 5-year OS between high B7H5 expression and low expression in the nucleus among patients with GC (28.0% vs 21.6%, $P = .254$), survival analysis showed that a high cytoplasmic B7H5 expression predicted poorer survival than a low cytoplasmic B7H5 expression in patients with GC (19.6% vs 37.5%, $P = .035$), while GC patients with high CD28H had poorer survival (39.4% vs 6.9%, $P = .002$). According to the expression of B7H5 in the cytoplasm and CD28H in immune cells, all patients were divided into four group, including low B7H5 and CD28H expressions (B7H5⁻CD28H⁻) group, low B7H5 expression with high CD28H expression (B7H5⁻CD28H⁺) group,

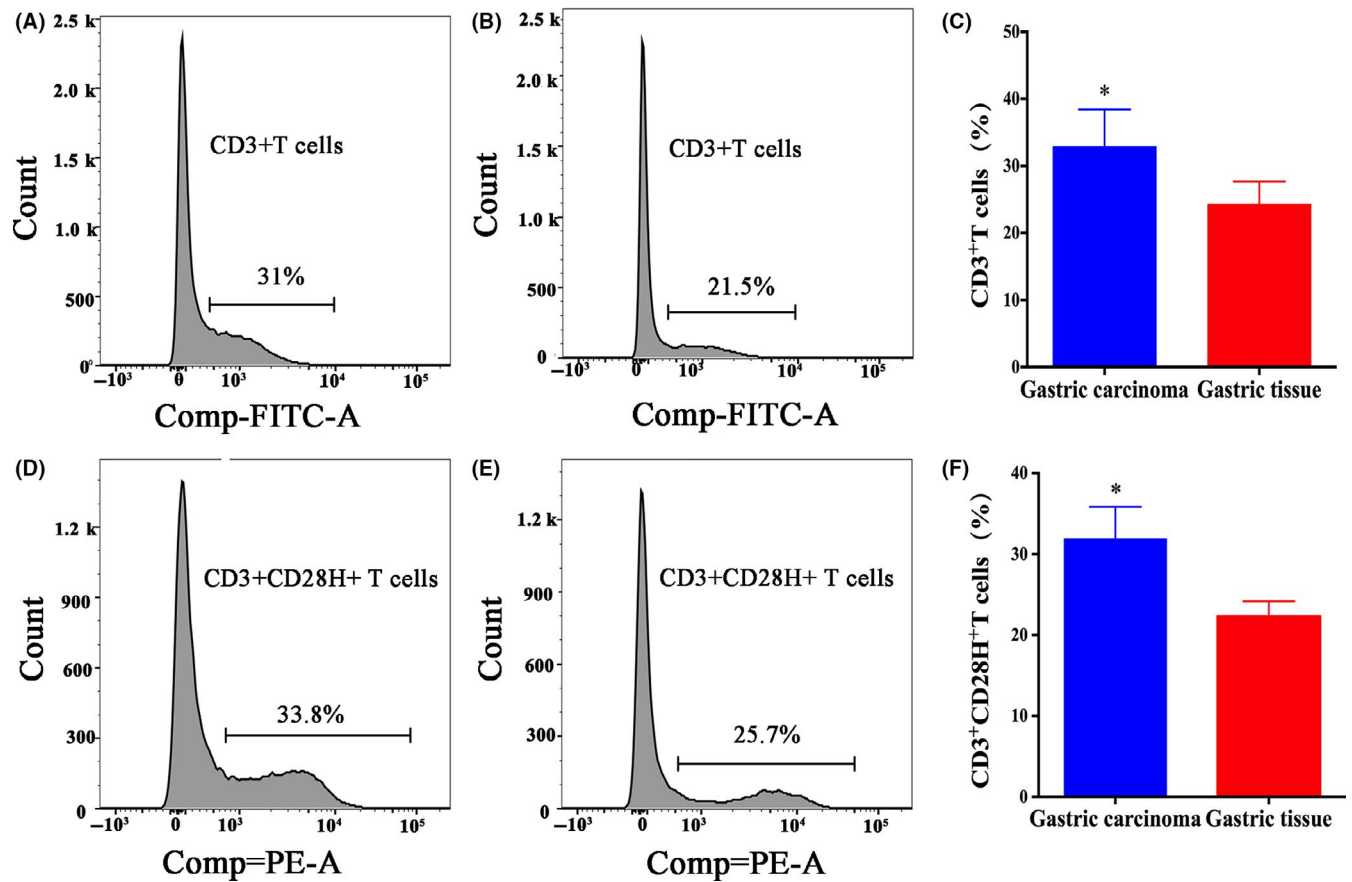


FIGURE 4 CD28H is expression on T cells in GC tissues and gastric tissues. A-B, Representative flow cytometry plots showing the count of CD3⁺T cells in GC tissues and gastric tissues. C, The mean percentages showed that the count of CD3⁺T cells in GC tissues was higher than that than gastric tissues, *, $P < .05$. D-E, Representative flow cytometry plots showing the count of CD3⁺CD28H⁺T cells in CD3⁺T cells in GC tissues and gastric tissues. F, The mean percentages showed that the count of CD3⁺CD28H⁺T cells in CD3⁺T cells in GC tissues was higher than that in gastric tissues, *, $P < .05$

high B7H5 expression with low CD28H expression (B7H5⁺CD28H⁻) group, and high B7H5 and CD28H expression (B7H5⁺CD28H⁺) group. The 5-year OS was 4.5% in the B7H5⁺CD28H⁺ group, 14.3% in the B7H5⁻CD28H⁺ group, 33.3% in the B7H5⁺CD28H⁻ group and 55.6% in the B7H5⁻CD28H⁻ group. There was a significant difference between B7H5⁺CD28H⁺ patients and B7H5⁻CD28H⁻ patients ($P = .001$), while there was no difference between B7H5⁻CD28H⁻ patients and B7H5⁺CD28H⁺ patients ($P = .111$), or between B7H5⁺CD28H⁻ and B7H5⁻CD28H⁻ patients ($P = .173$). There was a significant difference between B7H5⁺CD28H⁻ and B7H5⁺CD28H⁺ patients ($P = .006$), while there is no difference between B7H5⁺CD28H⁻ and B7H5⁻CD28H⁺ patients ($P = .777$). In addition, there was no difference between B7H5⁻CD28H⁺ and B7H5⁺CD28H⁺ patients ($P = .066$). However, the results of the chi-square test confirmed that there was no correlation between B7H5 expression and CD28H expression in patients with GC ($P = .844$). As listed in Table 4, significant prognostic factors for survival by univariate analysis were T stage, N stage, AJCC stage, B7H5 expression and CD28H expression. Multivariate analysis using the Cox proportional hazard model confirmed that B7H5 and CD28H expression levels were independent risk factors.

4 | DISCUSSION

The present study aimed to explore the clinical significance of the B7H5/CD28H pathway in GC and confirmed that the activities of B7H5 and CD28H might represent a new immunosuppressive mechanism within the GC microenvironment, as well as providing novel targets for GC immunotherapy. In the present study, we reported that B7H5 was overexpressed both in the cytoplasm and nucleus in tumour tissues from patients with GC compared with that in the adjacent non-cancerous tissues. In addition, there was no difference in 5-year OS between high B7H5 expression and low expression in the nucleus in patients with GC (28.0% vs 21.6%, $P = .254$), while high cytoplasmic B7H5 expression predicted poorer survival than did low cytoplasmic B7H5 expression in patients with GC (19.6% vs. 37.5%, $P = .035$). A study by Zhao et al showed that B7H5 was expressed in the cytoplasm of mouse embryonic fibroblast cells and could reduce the production of cytokines from T cells.¹⁵ This suggested that B7H5 expression in the cytoplasm might be important in immunotherapy. This was the same as the results of previous studies showing that B7H5 was expressed in the oesophagus, liver, colon and pancreas.^{12,16} However, a previous study by Shimono¹⁷ compared B7H5 expression in peripheral

TABLE 2 Correlation between B7H5 expression in the nucleus and clinicopathological characteristics

Variables	B7H5 expression		Total	χ^2	P-value
	High	Low			
Age (y)					
≤66	12	23	35	0.410	.522
>66	15	21	36		
T stage					
T1/T2	3	7	10	0.045	.831
T3/T4	24	37	61		
TNM stage					
I/II	15	17	32	1.935	.164
III/IV	12	27	39		
N stage					
N0	7	12	19	0.015	.901
N1/N2/N3	20	32	52		
M stage					
M0	26	41	67	0.001	.982
M1	1	3	4		
P53					
Negative	10	38	48	0.787	.375
Positive	7	16	23		
Sex					
Female	8	10	18	0.421	.516
Male	19	34	53		
Ki67					
Negative	11	21	32	0.330	.566
Positive	16	23	39		
Grade					
I/II	10	18	28	0.109	.741
III/IV	17	36	53		

^aStatistically significant ($P < .05$).

blood between 111 patients with untreated GC and 20 healthy volunteers. Their study showed that patients with GC had significantly higher B7H5 mRNA levels and higher B7H5 expression was associated with a better 5-year OS. This result revealed a different B7H5 expression pattern to that shown in the present study. We are unable to explain this difference because of the different methods and antibodies used to detect B7H5 expression between the two studies. However, we also showed that the expression of B7H5 was almost absent in B7H5^{KO}-BGC803 group in vivo. And it can also indicate the specificity of B7H5 (as showed in Figure S1). Other studies confirmed that high B7H5 expression was associated with poor prognosis in certain tumours. Janakiram et al¹⁸ showed that overexpression of B7H5 was associated with advanced stage of the disease and predicted high recurrent risk in breast cancer. In addition, Koirala et al¹⁹ confirmed that B7H5 was expressed in human osteosarcoma and was associated

TABLE 3 Differential expression of CD28H in cancerous and gastric tissues

Variables	CD28H expression		Total	χ^2	P-value
	High	Low			
Age (y)					
≤66	16	19	35	0.354	.552
>66	19	17	36		
T stage					
T1/T2	3	7	10	0.952	.329
T3/T4	32	29	61		
TNM stage					
I/II	13	19	32	0.063	.801
III/IV	22	17	39		
N stage					
N0	7	12	19	1.610	.205
N+	28	24	52		
M stage					
M0	35	32	67	2.296	.130
M1	0	4	4		
P53					
Negative	30	18	48	0.233	.630
Positive	13	10	23		
Sex					
Female	12	6	18	2.911	.088
Male	23	30	53		
Ki67					
Negative	15	19	32	0.700	.403
Positive	20	17	39		
Grade					
I/II	9	14	23	1.406	.236
III/IV	26	22	49		

^aStatistically significant ($P < .05$).

with metastases and worse survival. Our study also confirmed that B7H5 expression correlation with Ki67 expression in patients with GC ($P = .003$); Ki67 expression was detected in patients with GC with high B7H5 expression. Ki67 is an antigen associated with proliferation, and overexpression of Ki67 is negatively correlated with carcinoma differentiation.^{20,21} It further revealed that high B7H5 expression predicted poor outcome in patients with GC.

B7H5 has two receptors on T cells, including CD28H and another, as yet unknown, receptor. B7H5 has co-stimulatory and co-inhibitory effects against the immune response of T cells by CD28H and the unknown receptor.¹⁰ Therefore, we also examined the expression of CD28H. We found that the level of CD28H⁺ T cells in the tumour tissues in patients with GC was higher than that in the adjacent noncancerous tissues. Furthermore, patients in the B7H5⁺CD28H⁺ group had a lower 5-year OS compared with patients in the B7H5⁻CD28H⁻ group ($P = .001$). However,

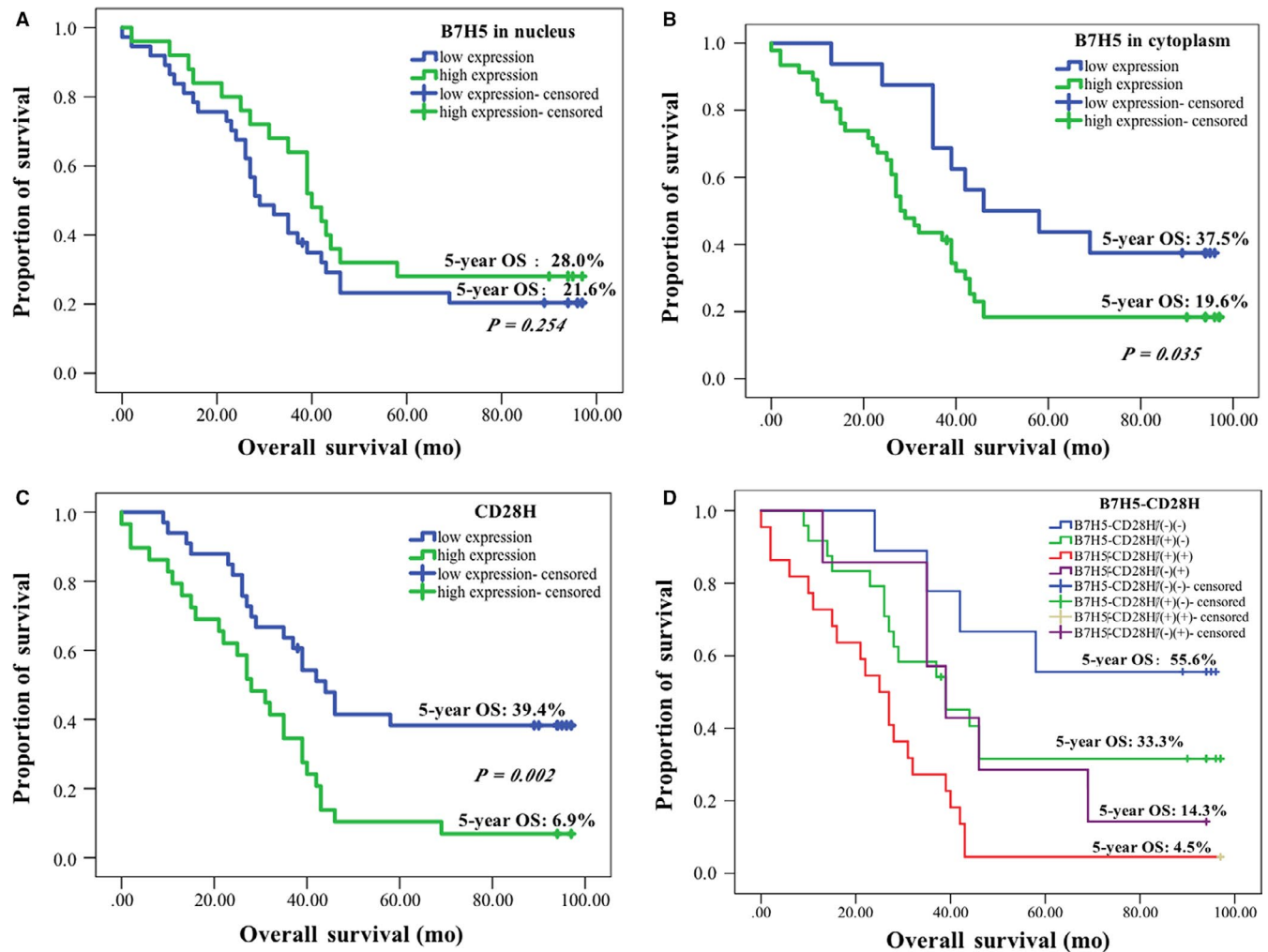


FIGURE 5 Correlation of B7H5/CD28H expression level and overall survival (OS) in GC patients. A, There is no difference in 5-year OS between high B7H5 expression and low expression in the nuclei in patients with GC (28.0% vs 21.6%, $P = .254$); B, High B7H5 expression predicted poorer survival than low B7H5 expression in patients with GC (19.6% vs 37.5%, $P = .035$); C, High CD28H expression predicted poorer survival than low CD28H expression in patients with GC (39.4% vs 6.9%, $P = .002$); D, Patients in the B7H5⁺CD28H⁺ group have a lower 5-year OS compared with patients in the B7H5⁻CD28⁻ group (4.5% vs 55.6%, $P = .001$), a significant difference was found in the 5-year OS between patients in B7H5⁺CD28H⁻ and B7H5⁻CD28H⁺ groups (33.5% vs 4.5%, $P = .006$), and there was no significant difference between the B7H5⁻CD28⁻ group and the B7H5⁻CD28H⁺ group (55.6% vs 33.3%, $P = .111$)

there was no significant difference between the B7H5⁻CD28H⁻ group and the B7H5⁻CD28H⁺ group ($P = .111$), while a significant difference was found in the 5-year OS between patients in B7H5⁺CD28H⁻ and B7H5⁺CD28H⁺ groups ($P = .006$). The results revealed that high expression of B7H5 and CD28H predict poor prognosis, especially when both are highly expressed, because of inhibition of the immune response of T cells. Moreover, B7H5 and CD28H acted as independent predictive factors in the overall survival of patients with GC. However, there was no correlation between B7H5 and CD28H expression ($P = .844$).

Our study showed that the B7H5/CD28H axis is a significant predictor of poor outcome. However, a new study by Yan et al showed that B7H5 is overexpressed in pancreatic ductal adenocarcinoma (PDAC) and high B7H5 expression is associated with better survival.²² Therefore, this study reminds us that different molecular mechanisms of B7H5 exist in different tumours. Some members of the B7/CD28

family have two opposing effects in different immune microenvironments.^{23,24} For example, B7H3 has a T-cell co-stimulatory and a co-inhibitory role in the immune response,²⁵⁻²⁷ similar to B7H5. B7H5 and CD28H are new members of the B7/CD28 family. The interaction between B7H5 and CD28H can promote the proliferation and cytokine production of T cells via the AKT pathway, while some studies confirmed that B7H5 could prevent the expression and secretion of cytokines by T cells to inhibit their response, including the IL-5, IL-10, IFN γ and TNF α .¹⁰ Therefore, the interaction of B7H5 and CD28H may inhibit the immune response as a co-inhibitor in GC.

In conclusion, we confirmed that B7H5 and CD28H expression levels are up-regulated and predict low survival in patients with GC, and are independent prognostic factors of overall survival. Although there is no correlation between B7H5 and CD28H expression, high expression of B7H5 and CD28H predicts poor prognosis, especially

TABLE 4 Prognostic factors in univariate and multivariate analyses

Factors	Univariate analysis	Multivariate analysis	
	P-value	P-value	HR (95%CI)
Gender			
Male vs Female	.747	.581	1.247 (0.570-2.726)
Age			
<67 vs ≤67	.326	.287	1.396 (0.756-2.578)
B7H5 expression			
Low vs High	.035 ^a	.016 ^a	2.575 (1.196-5.544)
CD28H expression			
Low vs High	.002 ^a	.017 ^a	2.337 (1.167-4.682)
T stage			
T2 vs T3, T4	.003 ^a	.192	2.758 (0.601-12.666)
N stage			
N0 vs N1, N2	.024 ^a	.426	1.561 (0.522-4.664)
M stage			
M0 vs M1	.273	.493	1.604 (0.415-6.199)
AJCC stage			
I, II vs III, IV	.001 ^a	.174	1.956 (0.744-5.141)
Pathological grade			
I, II vs III, IV	.887	.244	0.651 (0.316-1.340)

Abbreviations: CI, confidence interval; HR, hazard ratio.

^aStatistically significant ($P < .05$).

when both are highly expressed, via inhibition of the immune response of T cells. Therefore, the B7H5/CD28H axis could be an attractive target for GC immunotherapy.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

AUTHOR CONTRIBUTIONS

Xiangdong Cheng and Wei Chen contributed to conception or design of the work; Can Hu and Zhiyuan Xu contributed to drafting the work; Can Hu, Zhiyuan Xu, Shangqi Chen, Shaowei Mo, Chengwei Shi, Shenyu Wei, Liqiang Hu and Xiaofeng Wang contributed to data acquisition; Hang Lv and Yiping Wang contributed to data analysis; Xiang-dong Cheng and Can Hu contributed to supervision or mentorship. All the authors contributed important intellectual content for the overall work. Xiang-dong Cheng, Wei Chen and Zhi-yuan Xu take responsibility for the honesty and accuracy of the present study.

ETHICAL APPROVAL

The study was approved by the ethics committee of Zhejiang Cancer Hospital. The study conformed to the tenets of the Declaration of Helsinki. All patients provided written informed consent before taking part in the study.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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