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RESEARCH

B7-H6 Protein Expression has no Prognostic Significance in Human Gastric Carcinoma

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Abstract B7-H6, a novel member of the B7 family which binds to NKp30 to trigger antitumor NK cell cytotoxicity and cytokine secretion. Recently, B7-H family has been reported to be a negative regulator of the immune response in patients with gastric carcinoma. However, no reports have investigated the clinical significance of B7-H6 expression in human gastric cancer. We present the first study to the clinicopathological and prognostic value of B7-H6 in primary gastric tumors and adjacent non-tumor tissues at the protein level. Here we show that B7-H6 immunoreactivity was expressed in 6/60 (10 %) gastric tumors and 8/43 (18.60 %) adjacent non-tumor tissues. No statistical difference was found between B7-H6 expression and various prognostic factors; however, B7-H6-positive carcinomas were significantly associated with a higher differentiation (p =0.047). The survival analysis did not confirm the prognostic significance of B7-H6 expression in gastric cancer patients. Our data suggest that B7-H6, as detected by immunohistochemistry, is of limited value as a prognostic marker for gastric cancer.

Keywords B7-H6 · Gastric carcinoma · Adjacent non-tumor tissues · Immunohistochemistry

Introduction

Gastric carcinoma (GC) has continued to be a threat to human life. Gastric cancer is the third and fifth leading cause of cancerspecific mortality for men and women respectively, worldwide [1]. Diagnosis of GC at advanced stage is considered as a major reason for lower overall five-year survival rate . Therefore, considerable efforts will be made to explore novel biologic markers to benefit the early diagnosis of gastricl carcinoma.

In the past few years, members of the B7 family have been reported to control the T cell mediated immune response [2–6]. The regulatory signals yielded by the interaction between these B7 family members and their CD28 receptors on activated T cells have a powerful impact on the immune surveillance [2–6]. To date, several B7 family members have been investigated which are often overexpressed in gastric carcinoma, the B7-H6 molecule is a newly identified [7]. The absence of B7-H6 mRNA in normal tissues, coupled with its relative abundance among primary tumors and cell lines, indicates that its expression maybe correlate with tumor prognosis in a large cohort of patients . B7-H6 binds to NKp30, a human receptor which triggers antitumor NK cell cytotoxicity and cytokine secretion. Thus, functioning as a tumor-induced self-molecule, B7-H6 alerts innate immunity to cellular transformation via its interaction with the activating receptor NKp30 [7]. However, no reports have investigated the clinical significance of B7-H6 protein expression in patients with gastric cancer (Figs. 1 and 2).

The present study aims to investigate the expression of human B7-H6 protein in primary gastric tumors and adjacent non-tumor tissues by Immunohistochemistry (IHC), and identify the relationship between B7-H6 protein expression and clinicopathological findings, including prognosis in gastric cancer patients.

Materials and Methods

Patients and Specimens

Tumor samples and adjacent non-tumor tissues are collected from 103 patients, who were diagnosed as gastric cancer in

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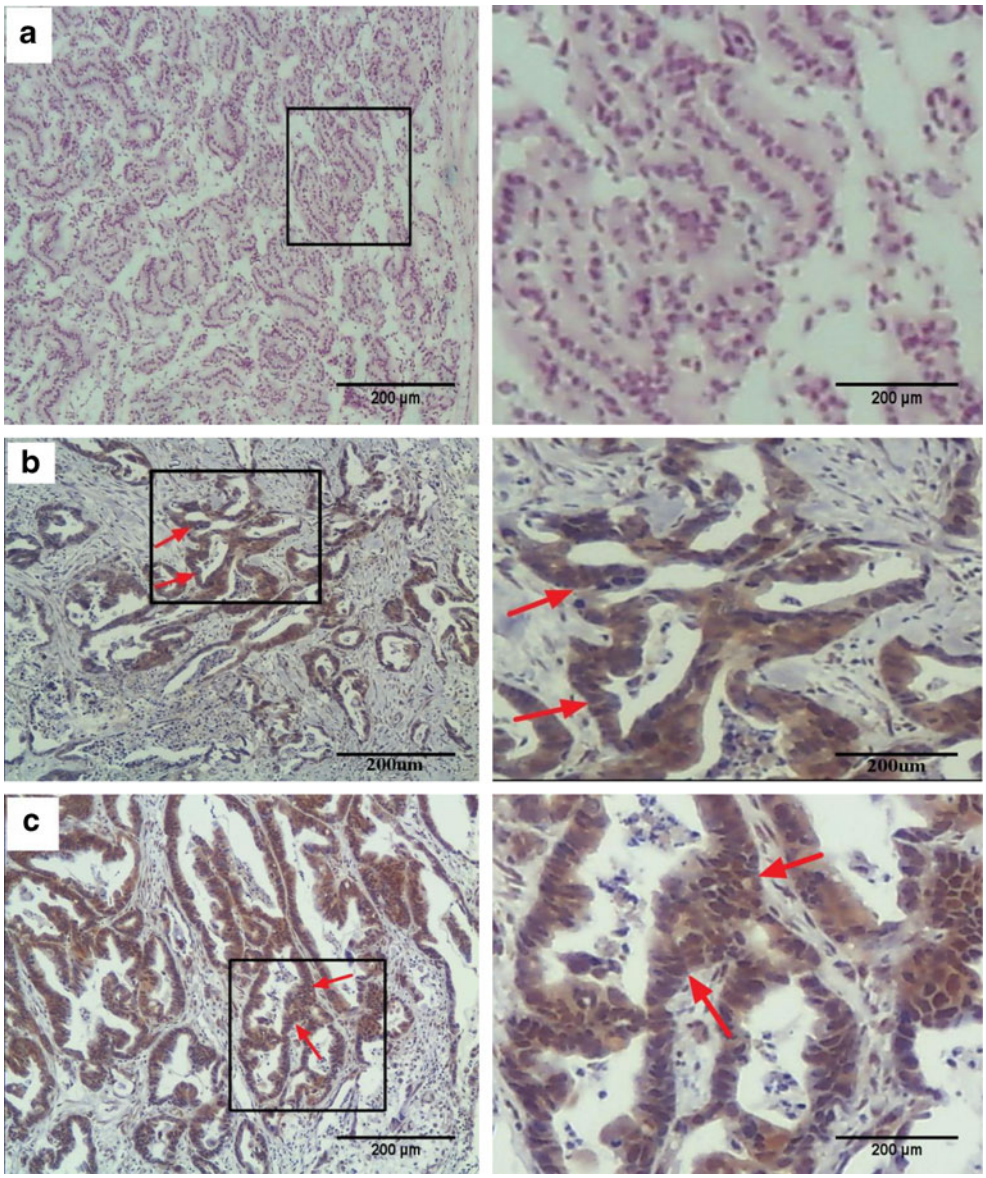

Fig. 1 Representative immunohistochemical staining of B7-H6 expression in gastric tumor tissue. Tumor cells with negative (**a**), weak (**b**) and moderate (**c**) expression of B7-H6. Scale bars indicate 200 um. Original magnification ×100 (*Left*); ×200 (*Right*)

general surgery department in the 1st affiliated hospital of Suzhou University between may 2008 and July 2009. No patients had received radiotherapy and chemotherapy before operation. Furthermore, none of the patients enrolled in this study had synchronous or metachronous cancer in other organs. These patients include 29 males and 74 females. Their median age was 59.3 (range: 38–84) years. According to gastric cancer TNM standard from Union for International Cancer Control (AJCC) (7th edition) [8], these gastric cancer patients were classified as 16 (I stage); 42 (II stage); 22(III stage); 23 (IV stage). The tissue samples were fixed in 10 % buffered formalin and embedded in paraffin. Patients were followed-up for 1–54 months. Informed consent was obtained before the study, and the protocol was approved by the Clinical Research Ethics Committee of the Suzhou University.

Immunohistochemistry

For B7-H6, 4 μm sections of tissue were mounted on superfrost slides. Slides were deparaffinized and rehydrated conventionally. Antigen retrieval was achieved by pressure cooking at 125 °C in 10 mmol/l citric acid (PH 6) for 3 min. The sections were incubated with B7-H6 antibody (Abcam, Cambridge, MA, USA) at 4 °C overnight diluted 1:50 in PBS. The reactions for B7-H6 were developed by the ABC method (Vectastain ABC kit, Vector Laboratories, Inc, Burlingame, CA) and visualized with diaminobenzidine tetrahydrochloride (10 min, at room temperature) and counterstained by Meyer's hematoxylin. Negative controls were treated with PBS without primary antibody under the same conditions. All immunostained slides were evaluated independently by two

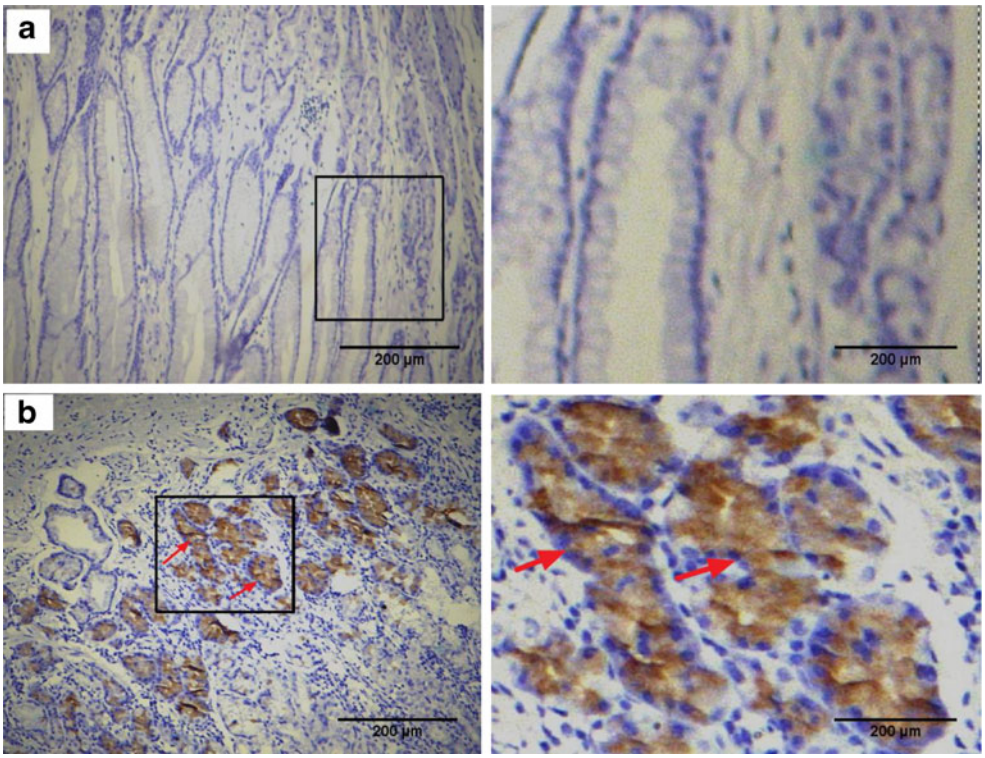

Fig. 2 Representative immunohistochemical staining of B7-H6 expression in adjacent normal gastric tissues. Cells with negative (**a**) and weak (**b**) expression of B7-H6. Scale bars indicate 200 um. Original magnification ×100 (*Left*); ×200 (*Right*)

pathologists. According to semiquantitative counting method, the degree of antibodies expression was evaluated by measuring both intensity and extent of staining: negative(−), 0-5 %; weakly positive (+), 6-25 %; moderately positive (++), 26-50 %; and strongly positive (+++), >50 %.

Statistical Analysis

Data were analysed by using SPSS 18.0 software. Chi^2 and Fisher's exact tests were used to analyze associations between immunohistochemical parameters of B7-H6 expression and clinicopathological features. Values of $p<0.05$ were considered significant.

Results

B7-H6 Protein Expression

In all tissues, immunoreactivity was found exclusively in the cytoplasm of cells. Although B7-H6 mRNA had been noted in a number of normal tissue, we detected B7-H6 protein expressed in adjacent normal gastric tissues . The B7-H6 protein expression was detected in 6/60 (10.00 %) tumors and 8/43 (18.60 %) adjacent normal gastric tissue. In tumor weak staining was 33 % (2/4) and intermediate staining was 67 % (4/6). While, adjacent normal gastric tissues were all weak staining (8/8). Strong staining was not detected in all specimens.

B7-H6 Protein Expression and Clinicopathological Factors

B7-H6 immunoreactivity presented no significant differences between the tumor tissue samples and the adjacent non-tumor ones. Whether gastric carcinomas or adjacent non-tumor tissues, no significant difference was found with respect to gender distribution, patient age, presence of distant metastasis at the time of diagnosis, tumor size, and histological classification. However, B7-H6-positive carcinomas were significantly associated with a higher differentiation ($p=0.047$) (Tables 1 and 2).

B7-H6 Protein Expression and Prognosis

The median overall survival times of B7-H6 negative and B7-H6 positive cases were 427 and 389 days respectively ($p>0.05$) (Table 1). B7-H6 gastric carcinomas were not statistically significantly associated with patient median overall survival. Similarly, the goup of adjacent normal gastric tissue was also not correlated with patient median overall survival. Their median overall survival times of B7-H6 negative and B7-H6 positive were 1,446 and 1,569 days respectively (Table 2).

Discussion

The B7 family and their receptor CD28 family of costimulatory and coinhibitory molecules have been intensely studied for their potential clinical impact in human malignancies. Members

Table 1 Relationship between B7-H6 expression and clinicopathological parameters in gastric carcinoma

Parameter	Patient(n)	B7-H6 (+)	Expression (−)	*p*
Age				1.000
<60	35	3	32	
≥60	25	3	22	
Gender				0.922
Male	44	5	39	
Female	16	1	15	
Tumor size(cm)				0.299
≥5	27	1	26	
<5	33	5	28	
Differentiation				0.047
High/middle	15	4	11	
Low/null	45	2	43	
Invasion				0.849
mucosa and muscle	17	1	16	
serosa	43	5	38	
Lymph node metastasis				0.110
Yes	42	2	40	
No	18	4	14	
Distant metastasis				0.320
Yes	15	3	12	
No	45	3	42	
TNM staging				0.224
I-II	34	2	28	
III-IV	26	4	26	
Survival time[a]				0.697
<427 days	16	2	14	
≥427 days	44	4	40	

[a] 427 days corresponds to the median survival time of the patients in the study

Table 2 Relationship between B7-H6 expression and clinicopathological parameters in adjacent normal gastric tissue

Parameter	Patient(n)	B7-H6 (+)	Expression (−)	*p*
Age				0.511
<60	21	3	18	
≥60	22	5	17	
Gender				0.239
Male	30	5	25	
Female	13	3	10	
Tumor size(cm)				1.000
≥5	19	3	16	
<5	24	5	19	
Differentiation				0.223
High/middle	16	1	15	
Low/null	27	7	20	
Invasion				0.689
mucosa and muscle	11	3	8	
serosa	32	5	27	
Lymph node metastasis				0.107
Yes	29	5	24	
No	14	3	11	
Distant metastasis				0.502
Yes	7	2	5	
No	36	6	30	
TNM staging				0.180
I-II	24	5	19	
III-IV	19	3	16	
Survival time[a]				0.600
<1,526 days	28	7	21	
≥1,526 days	15	1	14	

[a] 1526 days corresponds to the median survival time of the patients in the study

of the B7-H family have been shown to be overexpressed in gastric cancer, mostly with an adverse clinical association. For instance, B7-H1 (also known as PD-L1, B7-H, CD274) immunodetection could be used as an independent factor to evaluate the prognosis of gastric carcinoma. B7-H1 expression was found in 42.2 % of gastric carcinoma samples [9]. B7-H1 expression was significantly correlated to lymph node metastasis, tumor size, and depth of invasion, TILs infiltration [10, 11], whereas for B7-H2 (also known as PD-L2 and B7-DC) in situ expression data are available, and the clinical significance is unclear. Similarly, B7-H3 (also known as CD276 and B7RP-2) expression was an independent prognostic factor[12]. B7-H3 was expressed in 58.8 % samples of gastric carcinoma. B7-H3 immunolabeling was significantly enhanced, when the tumor infiltrated into the deep muscular layers, with tissue type or survival time of less than 2 years [13]. The level of expression of B7-H4 (also known as VTCN1, B7X, and B7S1) correlates to different clinical outcomes; high levels of B7-H4 expression have been shown to be positively associated with reduced patient's survival [14, 15]. However, no clinical relevance of B7-H6 expression was detected in gastric cancer.

To the best of our knowledge, we present the first study to date with respect to the clinicopathological and prognostic value of B7-H6 protein in a series of human gastric carcinomas and adjacent non-tumor tissues. A collection of 60 gastric carcinomas and 43 adjacent non-tumor tissues were compiled into tissue immunostained for B7-H6. B7-H6 immunoreactivity presented no significant differences between the tumor tissue samples and the adjacent non-tumor ones. The immunoreactivity of B7-H6 did not reveal any correlation with various clinicopathological and prognostic factors, such as age, sex, tumor differentiation, lymph node metastasis, TNM stage and distant metastasis. However, B7-H6-positive carcinomas were significantly associated with a higher differentiation ($p = 0.047$). The survival analysis did not confirm the prognostic significance of B7-H6 expression in gastric cancer patients.

Collectively, our findings suggest that B7-H6, as detected by immunohistochemistry, is of limited clinical value as a prognostic marker for gastric carcinoma, though the number of patients is small. But we cannot exclude that different results might be obtained in other tumor types. Therefore, the expression of B7-H6 has still to be investigated on a larger number of samples from different tumor types in different centers.

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Conflict of interest All the authors declare no conflict of interest.

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