Correlation between expression of B7-H1 and clinical progression in human esophageal carcinoma

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Abstract

Objective. To study the expression of B7-H1 in human esophageal carcinoma (EC) and its correlation with EC metastasis and prognosis. *Methods.* The immunohistochemical staining and RT-PCR were used to detect expressions of both B7-H1 protein and B7-H1 mRNA in 54 EC cases, and the relationship between the levels of B7-H1 protein expression and the clinical data about EC was analyzed. *Results.* The positive rate of B7-H1 protein expression in EC tissues was 48.15% (26/54), whereas it was absent in tumor adjacent normal esophageal tissues. The B7-H1 mRNA was demonstrated in all EC tissues, but absent in tumor adjacent normal esophageal tissues. The B7-H1 expression in EC tissues was related to the depth of neoplastic infiltration, lymph node metastasis and pTNM stage. *Conclusion.* The B7-H1 plays an important role in human EC progression, suggesting that it may be used as a new indicator to predict metastatic potential and prognosis of EC. [Life Science Journal. 2008; 5(4): 13 – 16] (ISSN: 1097 – 8135).

Keywords: human esophageal carcinoma; B7-H1; metastasis; prognosis

1 Introduction

China is a country with the highest morbidity and mortality of EC in the world, especially in the province of Henan. Most EC in China belongs to esophageal squamous cell carcinoma (ESCC); alternatively, the esophageal adenocarcinoma is dominant in western countries[1]. Current treatments, including surgery, chemotherapy and radiotherapy display low efficacy against EC. The ESCC always shows poor prognosis and the overall 5-year survival rate was only about 30%[1]. Therefore, it needs further study on the mechanism of ESCC and to develop new therapeutic strategies.

The evasion of tumor immunity plays an important role in carcinogenesis of ESCC, since the co-stimulatory molecules participate in regulating immune response. B7-H1 is a novel member of the B7 family molecules, while PD-1 is the receptor of B7-H1 to mediate the inhibition of activated T cell response[2,3]. The B7-H1 is aberrantly expressed in some tumor cell lines and several malignancies, which can mediate apoptosis of tumor-specific T-cell *in vitro*[4]. However, the expression of B7-H1 in ESCC with clinical significance has not been well documented. In this study we investigated the expression levels and localization of B7-H1 in ESCC tissue and analyzed the relationship between B7-H1 protein expression and clinical progression of ESCC i.e. the traditional clinicophathological variables.

2 Materials and Methods

2.1 Patients and samples

We examined 54 patients with EC who underwent surgery at the First Affiliated Hospital of Zhengzhou University, from September 2006 to July 2007. The patients received neither radiotherapy nor chemotherapy with median age 54.5 years old in a range of 40 to 69 years. The EC tissues were collected from the resected specimens, and the corresponding tumor adjacent normal esophageal tissues were obtained from 8 out of 54 EC cases. The his-

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topathological examination indicated that all tumors in this study were ESCC. Each tissue sample was cut into two aliquots, one aliquot was embedded into optimum cutting temperature compound (OCT, McCormick, England), frozen, and stored at – 80 ºC until use for immunohistochemistry. Another aliquot was snap-frozen in liquid nitrogen for isolation of total RNA.

2.2 Immunohistochemistry

Cryostat sections (5 μm) were fixed in 4% formaldehyde. The mouse antihuman B7-H1 mAb (Clone 5H1) which was kindly provided by Dr. Shengdian Wang (Center of Infection and Immunity, Institute of Biophysics, The Chinese Academy of Sciences), and Histostain-Plus kit (Zymed, US) was used for immunohistochemical staining according to the manufacture's instructions, and the slides were counterstained with hematoxylin. The negative controls were set by mouse IgG1 (eBioscience, US) substituted for the primary antibody.

2.3 Immunohistochemical staining

The B7-H1 immunoreactivity (IR) located in the cytoplasm or on the membrane was defined as the percentage of tumor cells by counting the number of B7-H1-IR tumor cells among 1000 tumor cells in at least five fields randomly selected tumor foci in each section under high magnification (× 400). The specimens with more than 10% B7-H1-IR tumor cells were taken as positive[5].

2.4 Extraction of RNA and semi-quantitative RT-PCR

The total RNA was isolated from the esophageal tissues with Trizol reagent (Invitrogen, USA) and reversely transcribed to cDNA using a single reverse transcriptase synthesis step with Superscript reagents (Invitrogen Life Technologies). The PCR was conducted in 25 μl of reaction mixture which consisted of 0.5 μl cDNA template, 0.5 U Taq DNA polymerase, 2.5 mM dNTP mixture, 2.5 μl 10 × PCR buffer, and 50 pM sense and antisense primers, besides, the β-actin was used as an internal control. Respective sequences of sense and antisense primers, and the expected product sizes were as follows: for β-actin (409 bp): 5'-CAACTGGGATGACATGGAGAACCTTGATGTCACGCACGATTT-3'; for B7-H1 (594 bp): 5'-GACCTATATGTGGTAGAGTATGGTAGCTTCAGCTGTATGGTTTTCCTCAGGATC-3'. The PCR conditions were as follows: initial denaturing at 95 ºC for 3 minutes, followed by 35 thermal cycles consisted of denaturation at 95 ºC for 30 seconds, annealing at 54 ºC for 1 minute, and then 72 ºC for 1 minute; a final extension at 72 ºC for 5 minutes. After PCR amplification, the PCR product was observed in 1% agarose gel.

2.5 Statistical analysis

The statistical analysis was performed by using the SPSS 13.0 software and the B7-H1-IR positive rates by χ^2 test and correlations as *r* were evaluated. The significance level was set at 0.05, and all *P* values were two-sided.

3 Results

3.1 *In situ* expression of B7-H1-IR in human ESCC

By immunohistochemistry, the B7-H1-IR protein localized in the cell membrane, cytoplasm, or both, in a focal or scattered pattern (Figure 1A) in 26 of 54 (48.15%) ESCC specimens. In contrast, no B7-H1-IR was found in the normal esophageal tissues (Figure 1B).

3.2 B7-H1 mRNA expression in human ESCC

As shown in Figure 2, the B7-H1 mRNA expression was detected by RT-PCR in the ESCC tissue, but negative in corresponding adjacent normal esophageal tissue.

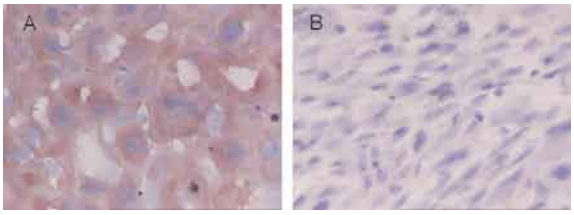

Figure 1. Immunohistochemical staining of human esophageal tissue for B7-H1. Representative case of B7-H1-IR positive in ESCC tissue (A) and B7-H1-IR negative (B) in tumor adjacent normal esophageal tissue, counterstaining with hematoxylin (× 400).

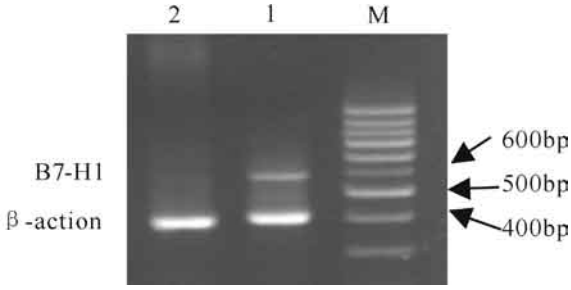

Figure 2. B7-H1 expression by RT-PCR. Representative determination of mRNA levels of B7-H1 (594 bp) and β-actin (409 bp) in the esophageal tissue. M: Molecular mass marker; Lane 1: B7-H1 mRNA in ESCC tissue; Lane 2: B7-H1 mRNA negative in the adjacent normal esophageal tissue.

3.3 Correlations between B7-H1 protein expression and clinicopathological parameters of ESCC patients

Table 1. Correlations between B7-H1 protein expression and clinicopathological parameters of ESCC patients

Variables	*n*	B7-H1 positive		$\bar{X}$	*P*
		n	%		
Sex					
Male	37	18	48.6	0.012	0.914
Female	17	8	47.1		
Age					
≥ 55 years	36	20	55.6	2.374	0.123
< 55 years	18	6	33.3		
Tumor location					
Upper 1/3	21	13	61.9		
Middle 1/3	16	7	43.8	2.841	0.242
Lower 1/3	17	6	35.3		
Differentiation					
High /medium	36	16	44.4	0.593	0.441
Low	18	10	55.6		
Tumor size					
< 5 cm	35	17	48.6	0.007	0.933
> 5 cm	19	9	47.4		
Depth of invasion					
Not to the muscularis	23	7	30.4	5.035	0.025
To the muscularis	31	19	61.3		
Lymph node metastasis					
Without	29	8	27.6	10.608	0.001
With	25	18	72.0		
Distal metastasis					
Without	16	4	25.0	4.880	0.027
With	38	22	57.9		
TNM stage					
I – II	22	6	27.3	8.984	0.003
III – IV	32	22	68.8		

Table 1 showed that the B7-H1 protein expression in ESCC was significantly correlated with depth of invasion, lymph node metastasis, distal metastasis, and pTNM III – IV ($P < 0.05$). Moreover, There was no relationship found between B7-H1-IR and sex, age, tumor location, differentiation, and tumor size in the ESCC patients ($P > 0.05$).

4 Discussion

The tumor evasion from immune response through circulating pathway has been reported and the B7 family molecules are one of hot spots in this area. B7-H1, a novel member of the B7 family of costimulatory molecules, plays an important role in regulating T and B cells activation[2,6,7]. It was confirmed that B7-H1 mRNA was also expressed in non-lymphoid tissue, in addition to expression in the lymphoid tissue[2,3,6]. Besides the macrophage lineage, the normal human tissues do not express B7-H1; in contrast, the B7-H1 is abundant in human carcinomas of lung, ovary, colon, and in melanomas[8,9]. Some researches indicated that B7-H1, as the ligand on the tumor cells, interacted with PD-1, as the receptor on activated T cells to inhibit Th1-biased responses. In addition, the expression of B7-H1 on tumor cells could induce apoptosis of activated T cells and facilitate the tumor evasion from immune response[10].

In the present study, we demonstrated that B7-H1 expressed in ESCC tissue in both protein and mRNA levels, but not in adjacent normal esophageal tissue, as corrob-

orate results in other malignancies showing that B7-H1 on tumor cells might contribute to negative regulation of immune response and promote tumorigenesis. The ESCC is one of the most common cancers in China, with poor prognosis, and most patients with ESCC die of tumor infiltration and metastasis. Our results also indicated that the levels of B7-H1 in ESCC were statistically correlated with lymph node metastasis, distant metastasis, infiltration depth, and pTNM III – IV, implying that the tumor may be in advanced stage with poor prognosis and the B7-H1, may be a useful biomarker for judgement of human ESCC prognosis. In addition, this study strongly suggested that B7-H1 might be one immunotherapeutic target for human ESCC in near future.

5 Conclusion

The B7-H1 plays an important role in human EC progression, suggesting that it may be used as a new indicator to predict metastatic potential and prognosis of EC.

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