J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-009-0116-2
29 (1): 77-79, 2009

Costimulatory Molecule B7-H1 on the Immune Escape of Bladder Cancer and Its Clinical Significance*

Yonghua WANG (王永华), Qianyuan ZHUANG (庄乾元), Siwei ZHOU (周四维), Zhiquan HU (胡志全), Ruzhu LAN (兰儒竹)
Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Summary：B7-H1, a recently described member of the B7 family of costimulatory molecules, is thought to be involved in tumor immune escape by inducing T-cell apoptosis. In order to investigate the relationship between B7-H1 and immune escape of bladder cancer, B7-H1 expression in 50 cases of bladder cancer was detected by using immunohistochemical method. Survival curves were constructed using the Kaplan-Meier method and independent prognostic factors were evaluated using the Cox regression model. Our results showed that the positive rate of B7-H1 immunostaining in normal bladder tissue and bladder cancer was 0 and 72% respectively. The expression of B7-H1 was strongly associated with the pathological grade, clinical stage and recurrence (P<0.05). The survival rate was significantly lower in patients with B7-H1 positive group than in those with B7-H1 negative group and multi-variable analysis revealed that B7-H1 could be regarded as an independent factor in evaluating the prognosis of bladder cancer. It is concluded that the expression of B7-H1 is strongly associated with neoplastic progression and prognosis of bladder cancer. The manipulation of B7-H1 may become a beneficial target for immunotherapy in human bladder cancer.
Key words：bladder neoplasm; costimulatory molecule; B7-H1; immune escape

Cancer progression has been attributed to a variety of immune escape strategies. These include down-regulation of cell-surface MHC class I molecules, secretion of immunosuppressive factors, and lack of T-cell costimulation[1-3]. B7-H1 (also known as PD-L1) is a recently discovered T-cell costimulatory molecule that has been implicated as an important molecule in tumor immune escape. It can inhibit immune responses by inducing T-cell apoptosis, impairing cytokine production, and diminishing the cytotoxicity of activated T cells[4-6]. In this study, we detected the expression of B7-H1 in bladder cancer by immunohistochemical method for exploring the relationship between B7-H1 and immune escape of bladder cancer.

1 MATERIALS AND METHODS

1.1 Clinical Data

Specimens were collected from 50 patients with bladder cancer who had undergone surgical resection at Tongji Hospital, Tongji Medical College, HUST (China), between 2000 and 2002. All the specimens of bladder cancer had been identified pathologically. The patients included 40 males and 10 females with age ranging from 42 to 78 years (average 61.7 years). There were 34 patients with primary bladder cancer and 16 patients with recurrent bladder cancer. According to the WHO and UICC standard, tumor grade was G_1-G_2 in 23 (low grade group) and G_3 in 27 patients (high grade group), and the stage was $T_{a\text{-}1}$ in 19 (superficial group) and $T_{2\text{-}4}$ in 31 patients (invasive group). The median follow-up duration for all patients was 27.94 months, with a range of 6-52 months. Besides, 10 samples from normal bladder mucosa served as control group.

1.2 Immunohistochemistry

Immunohistochemical staining was performed on 5-μm thick sections of the tissue microarray blocks. Paraffin-embedded sections were mounted on Superfrost glass slides, deparaffinized, rehydrated in a graded ethanol series, and then subjected to microwave antigen retrieval. Endogenous peroxidase activity was blocked by using 3% hydrogen peroxide. Sections were incubated at 4°C overnight with rabbit anti-human B7-H1 polyclonal antibody (Santa Cruz Company, USA) diluted to 1:50. Immunohistochemical staining was performed according to the instruction of SABC kit (Boster Company, China). Sections were then counterstained with hematoxylin and then dehydrated, cleared and mounted.

To evaluate the specificity of the reaction, PBS was used for replacing the primary antibody as negative control. The positive expression of B7-H1, which was predominantly found in the cytoplasm or on the membrane, was light yellow to brown. The percentage of B7-H1 positive tumor cells among the total number of tumor cells was scored in 3 randomly selected high-power (×400) fields and the percentage >10% was classified as B7-H1 positive.

1.3 Statistical Analysis

All of the analyses were performed by using SPSS 13.0 software. The chi-square test was used for group comparison. The Kaplan-Meier method was used to estimate the probability of survival, and significance was

Yonghua WANG, e-mail: doctorwangyonghua@yahoo.cn
*This project was supported by a grant from Hubei Provincial Science and Technology Key Program Foundation of China (No. 2007AA402C60).

assessed by the Log-Rank test. Multivariate analysis was done using the Cox regression model in stepwise method. *P* values of less than 0.05 were considered to be statistically significant.

2 RESULTS

2.1 Expression of B7-H1 in Normal Bladder Tissue and Bladder Cancer

The positive rate of B7-H1 immunostaining in the specimens of normal bladder tissue and bladder cancer were 0 (0/10) and 72% (36/50) respectively. The chi-square test showed that there was significant difference in the B7-H1 expression between bladder cancer and normal bladder tissues (*P*=0.000). Staining intensity in the cytoplasm or on the membrane ranged from light yellow to brown (fig. 1).

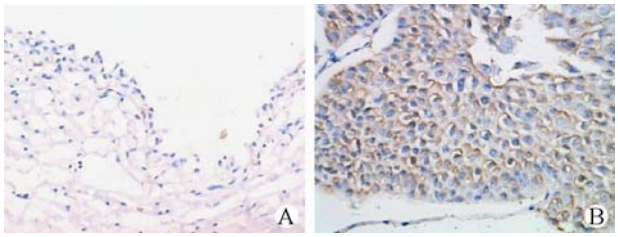

Fig. 1 Expression of B7-H1 in normal bladder tissue (A) and bladder cancer tissue (B) (SABC×400)

2.2 Correlation of B7-H1 Expression with the Biological Characters of Bladder Cancer

The correlation of B7-H1 expression with the biological characters of bladder cancer was summarized in table 1. It was observed that the B7-H1 expression was not associated with gender of patients, but there was a significant association between B7-H1 expression and pathological grade and clinical stage. Meanwhile, the positive rate of the recurrent cases was higher than that of the primary cases and the difference was statistically significant (table 1).

Table 1 Correlation of B7-H1 expression with the biological characters of bladder cancer

Items	*n*	Positive (%)	*P* values
Gender			
Male	40	31 (77.5)	0.181
Female	10	5 (50.0)	
Grade			
Low grade group	23	12 (52.2)	0.004
High grade group	27	4 (88.9)	
Stage			
Superficial group	19	10 (52.6)	0.017
Invasive group	31	26 (83.9)	
Primary and Recurrent			
Primary group	34	21 (61.8)	0.044
Recurrent group	16	15 (93.8)	

2.3 Correlation of B7-H1 Expression with the Prognosis of Bladder Cancer

Survival time was calculated from the date of surgery to the date of death. The Kaplan-Meier survival curve indicated that the survival rate in patients with B7-H1 positive group was significantly lower than that in those with B7-H1 negative group and the difference was statistically significant (*P*=0.020, fig. 2).

To determine the prognostic value of B7-H1 expression, we did multivariate analysis using Cox regression model to study 5 factors (age, gender, pathological grade, clinical stage, B7-H1 expression). Pathological grade, clinical stage and B7-H1 expression had significant prognostic values. Among the total, the relative risk of B7-H1 expression was 2.24 and 95% confidence interval was 1.16-4.38 (*P*=0.011). The multivariate analysis revealed that B7-H1 could be regarded as an independent factor in evaluating the prognosis of bladder cancer.

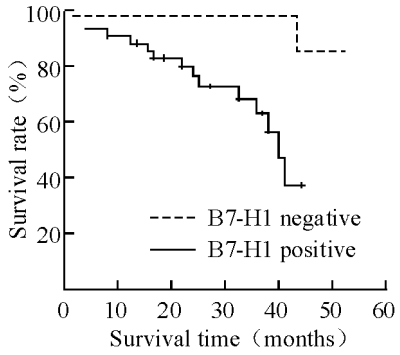

Fig. 2 Correlation of B7-H1 expression with the prognosis of bladder cancer

3 DISCUSSION

Interactions between the immune system and malignant cells play an important role in tumorigenesis. Failure of the immune system to detect and reject transformed cells may lead to tumor development and tumors can use multiple mechanisms to escape from immune-mediated rejection[7-9]. B7-H1 is a cell-surface glycoprotein within the B7 family of T-cell costimulatory molecules. Constitutive expression of B7-H1 is normally restricted to macrophage-lineage cells[10, 11]. In contrast, several human cancers, including breast cancer, ovarian cancer, lung cancer, colon cancer, kidney cancer, lymphoma, and melanoma, have now been reported to aberrantly express B7-H1[12-15]. Tumor cell expression of B7-H1 had been shown to inhibit immune response by either binding to programmed death-1 (PD-1) or a putative non-PD-1 receptor on the surface of T lymphocytes to induce antigen-specific T-cell apoptosis or anergy[16]. Because T lymphocytes play a central role in mediating acquired antitumoral immunity, it has been proposed that expression of B7-H1 may endow tumors with a mechanism to evade host immune destruction.

In this study, we used immunohistochemical technique to detect the expression of B7-H1 in normal bladder tissue and bladder cancer. It was observed that there was no B7-H1 expression in normal bladder tissue but there was a substantial expression of B7-H1 in bladder cancer and the expression of B7-H1 was strongly associated with the pathological grade, clinical stage and recurrence of bladder cancer. It indicates that B7-H1 which has the recognized ability to inhibit T-cell mediated an-

titumor immunity, may contribute to the pathogenesis and progression of bladder cancer.

Another interesting observation from our study was that the expression of B7-H1 was strongly associated with the postoperative prognosis in bladder cancer. The survival rate was significantly lower in patients with B7-H1 positive group than in those with B7-H1 negative group. And the analysis of multi-variable reveals that B7-H1 can be regarded as an independent factor in evaluating the prognosis of bladder cancer. This result indicates that B7-H1 can be regarded as an important prognosis factor in human bladder cancer.

Moreover, our study had implications for the design of T-cell-based immunotherapy. For example, adoptive immunotherapy strategies require the infusion of pre-activated T cells. If confronted by an apoptotic molecule such as B7-H1, tumor-specific T cells would be deleted selectively at the tumor site[17, 18]. Therefore blockade of B7-H1 by specific monoclonal antibodies or soluble inhibitors might enhance cytotoxic T lymphocyte killing of established cancers. And recent *in vivo* studies have demonstrated that antibody-mediated blockade of PD-L1 is capable of facilitating the rejection of certain PD-L1-expressing tumors in several murine models[19, 20]. So our findings indicate that PD-L1 may serve as a rational immunotherapeutic target for the treatment of bladder cancer as well as a viable prognostic marker to predict bladder cancer risk.

In conclusion, we have demonstrated the remarkable expression of B7-H1 in bladder cancer. The B7-H1 expression in tumor cells was well associated with pathological grade, clinical stage, recurrence and the postoperative prognosis of bladder cancer. These results suggest that the expression of B7-H1 may contribute to the neoplastic progression and poor prognosis of bladder cancer. The basis for these associations may relate to the recognized ability of B7-H1 to involve in tumor immune escape by inducing T-cell apoptosis. Therefore, the manipulation of B7-H1 may become a beneficial target for immunotherapy in human bladder cancer.

REFERENCES

1 Igney FH, Krammer PH. Immune escape of tumors: apoptosis resistance and tumor counterattack. J Leukoc Biol, 2002,71(6):907-920

2 Pardoll D, Allison J. Cancer immunotherapy: Breaking the barriers to harvest the crop. Nat Med, 2004,10(9):887-892

3 Lyman MA, Aung S, Biggs JA, *et al*. A spontaneously arising pancreatic tumor does not promote the differentiation of naive $CD8^+$ T lymphocytes into effector CTL. J Immunol, 2004,172(11):6558-6567

4 Dong H, Strome SE, Salomao DR, *et al*. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nat Med, 2002, 8(8):793-800

5 Junko H, Mingcong W, Megumi M, *et al*. B7-H1-Induced Apoptosis as a Mechanism of Immune Privilege of Corneal Allografts. J Immunol, 2006,177(21): 5928-5935

6 Fumihiko T, Kae T, Noriko O, *et al*. Predominant expression of B7-H1 and its immunoregulatory roles in oral squamous cell carcinoma. Oral Oncol, 2006,42(3): 268-274

7 Wintterle S, Schreiner B, Mitsdoerffer M, *et al*. Expression of the B7-Related Molecule B7-H1 by Glioma Cells: A Potential Mechanism of Immune Paralysis. Cancer Res, 2003,63(21):7462-7467

8 Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol, 2007,19(7): 813-824

9 He L, Zhang G, He Y, *et al*. Blockade of B7-H1 with sPD1 improves immunity against murine hepatocarcinoma. Anticancer Res, 2005,25(5):3309-3313

10 Dong H, Zhu G, Tamada K, *et al*. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat Med, 1999, 5(12):1365-1369

11 Brown JA, Dorfman DM, Ma FR, *et al*. Blockade of programmed death 1 ligands on dendritic cells enhanced T cell activation and cytokine production. J Immunol, 2003,170(3):1257-1266

12 Konishi J, Yamazaki K, Azuma M, *et al*. B7-H1 expression on non-small cell lung cancer cells and its relationship with tumor-infiltrating lymphocytes and their PD-1 expression. Clin Cancer Res, 2004,10(15):5094-5100

13 Ghebeh H, Mohammed S, Al-Omair A, *et al*. The B7-H1 (PD-L1) T lymphocyte- inhibitory molecule is expressed in breast cancer patients with infiltrating ductal carcinoma: correlation with important high-risk prognostic factors. Neoplasia, 2006,8(3):190-198

14 Strome SE, Dong H, Tamura H, *et a1*. B7-H1 blockade augments adoptive T-cell immunotherapy for squamous cell carcinoma. Cancer Res, 2003,63(19): 6501-6505

15 Thompson RH, Kuntz SM, Leibovich BC, *et al*. Tumor B7-H1 is associated with poor prognosis in renal cell carcinoma patients with long-term follow-up. Cancer Res, 2006,66(7):3381-3385

16 Das S, Suarez G, Beswick EJ, *et al*. Expression of B7-H1 on gastric epithelial cells: its potential role in regulating T cells during Helicobacter pylori infection. J Immunol, 2006, 176(5):3000-3009

17 Blank C, Kuball J, Voelkl S, *et al*. Blockade of PD-L1(B7-H1) augments human tumor-specific T cell responses in vitro. Int J Cancer, 2006,119(2):317-327.

18 Hirano F, Kaneko K, Tamura H, *et al*. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. Cancer Res, 2005, 65(3): 1089-1096.

19 Yoshsiko I, Masayoshi I, Yoshimasa T, *et a1*. Involvement of PD-LI on tumor cells in the escape from host immune system and tumor immunotherapy by PD-LI blockade. Proc Natl Acad Sci USA, 2003,99(19): 12293-12297

20 Curiel TJ, Wei S, Dong H, *et al*. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. Nat Med, 2003,9(5):562-567

(Received June 27, 2008)