Expression of B7 Homolog 1 (B7H1) Is Associated with Clinicopathologic Features in Urothelial Bladder Cancer

Background: B7 homolog 1 (B7H1) plays an important role in regulating tumor immunity. The purpose of this study was to probe the relationship between B7H1 expression and clinical outcomes in urothelial bladder cancer.

Material/Methods: We investigated 110 urothelial bladder cancer cases. The expressions of B7H1 in tumors were analyzed by immunohistochemistry and RT-PCR. The correlation between B7H1 expression and survival rate was analyzed by log-rank test.

Results: B7H1 expression was significantly increased in cancerous tissues compared to normal tissues (p<0.05). B7H1 expression was not associated with sex, age, diameter, or the combination of these factors (p>0.05). The positive expression of B7-H1 was positively correlated with grade, stage, recurrence, and metastasis (p<0.05). The RT-PCR results were consistent with the immunohistochemistry outcomes. Furthermore, the expression of B7H1 in tumors was highly correlated with the survival rate (p<0.05).

Conclusions: Expression of B7H1 is correlated with clinicopathologic features in bladder cancer. Up-regulation of B7H1 can result in progression and recurrence of urothelial bladder cancer.

MeSH Keywords: Antigens, CD274 • Survival Analysis • Urinary Bladder Neoplasms
Background

Urothelial bladder cancer (UBC) is a common disease, accounting for approximately 7% of all cancer cases [1,2]. Transurethral resection combined with intravesical chemotherapy is the standard treatment for Ta and T1 disease in urothelial bladder cancer, but the outcomes remain poor [3]. Some studies have shown that the clinical outcome of UBC is associated with immune responses in cancer patients with anti-immune cells [4,5]. Response to therapy can be predicted by the combination of clinical outcomes and tissue-based molecular markers, which could help in administering the optimal local and systemic therapy in UBC patients.

B7 homolog 1 (B7-H1), also known as programmed death ligand 1 (PD-L1), is the third member of the B7 family [5]. T cells are immune cells that play a critical role in mediating anti-tumor immunity. A large numbers of T cells are present in bladder cancer, and the cells positively control the local anti-tumor responses, such as CD4+ T cells and CD86 [6]. B7-H1 can inhibit proliferation of T cells and is a negative stimulus in the process of activation of T cells through suppressing the secretion of some cytokines, for example, interferon-γ (IFN-γ), interleukin-10 (IL-10), IL-14, and IL-2 [7,8]. Expression of B7-H1 was reported to be higher in tumor tissues and the number of immune cells in peripheral blood was found to be related with tumor escape [9]. Up-regulation of B7-H1 has been reported to be correlated with progression of thyroid cancer, lung cancer, ovarian cancer, colon cancer, and melanoma [10–14]. A previous study showed that B7-H1 expression is involved in the mitogen-activated protein kinase (MAPK) pathway, especially the extracellular signal-regulated kinases (ERK) pathway in bladder cancer [15]. In the in vitro lung cancer microenvironment, B7H1 expression was up-regulated, with increasing transforming growth factor β (TGF-β) and was associated with regulatory T cell generation [16]. Moreover, overall survival was correlated with B7H1 expression in patients with UBC. These findings suggest that B7H1 plays an integral role in the immunological escape mechanism in UBC.

Many studies showed that B7H1-positive expression was associated with rapid cancer progression. The expression of B7H1 is significantly associated with the clinicopathological variables and postoperative survival in esophageal cancer [17]. Patients with high B7H1 expression show shorter survival than those with low B7H1 expression.

In this study, we investigated the B7H1 expression in UBC patients and analyzed the relationship between expression of B7H1 and clinicopathological features. The results may contribute to clinical treatment of UBC.

| Table 1. The clinicopathologic parameters of the 98 cases. |
|-----------------------------------------------------------|
| **Age (y)**       | **Metastatic tumor** | **n** |
| <65              | Metastasis           | 46    |
| ≥65              | Non-metastasis       | 52    |
| **Tumor grade**  | **Tumor size (diameter)** | **n** |
| I                | <3 cm                | 23    |
| II               | ≥3 cm                | 46    |
| III              | Focus                | 29    |
| **Tumor stage**  | **Solitary**         | 45    |
| Ta–T1            | Multiple             | 25    |
| T2–T4            |                      | 73    |
| **Tumor type**   |                        | 57    |
| Primary          | Recurrence           | 41    |

Material and Methods

Clinical study

We enrolled 98 patients (22 females and 76 males) who underwent bladder urothelial cancer surgery at Yantai Affiliated Hospital of Binzhou Medical University from 1 September 2009 to 1 October 2011. We obtained cancerous tissue samples from each patient, as well as 12 tissue samples adjacent to tumors. None of the patients in this study had received any radiation therapy or chemotherapy before the operation. All patients were diagnosed by the method of cystoscopy, ultrasound, computerized tomography urography (CTU), and pathology. Written permission was obtained from the patients prior to surgery. All patients were confirmed with pathological examination. All tissues were stored in a liquid nitrogen tank after surgical resection.

The tumors were classified according to the tumor-node-metastasis (TNM) staging system of the International Union Against Cancer (1997) and the World Health Organization (WHO). All clinicopathologic parameters are summarized in Table 1.

This study was approved by Medical Ethics Committee of Binzhou Medical University.

Immunohistochemistry

Bladder cancer tissues and tissue adjacent to bladder cancer were fixed with 4% paraformaldehyde (Beijing North of Conde Clinical Reagent Co., Beijing, China), embedded in paraffin, and
then sliced into 4-μm sections. All sections were incubated for 4 h at room temperature with anti-B7H1 antibody (1: 50, Boster, China). Phosphate-buffered saline (PBS) was used as a blank control to replace the first antibody. The second antibody was diluted to 1: 500. Positivity of cell plasma or membranes was indicated by tan color. Three randomly selected fields were observed at high magnification (*400). Greater than 10% positive cells was regarded as positive. The results were analyzed using AlphaView SA software (Thermo Fisher Scientific, Waltham, MA, USA).

RT-PCR

Total RNA was extracted from fresh urothelial bladder cancer tissue. The mRNA was reverse-transcribed into first-strand cDNA using M-MLV reverse transcriptase (Promega, USA). PCR reactions were carried out using specific primers for B7H1 (sense: 5′-GCCGACTACAAGCGAATTAC-3′, antisense: 5′-TCTCAGTGTGCTGTCACAT-3′, product length 233 bp). β-actin served as the internal control (sense: 5′-CGGGAATCGTGCGTGAC-3′, antisense: 5′-TAGAAGCATTTGCGGTGG-3′, product length 511 bp). The cycling parameters were: 94°C for 3 min, 90°C for 30 s, and 55°C for 30 s. The extension was 1 min at 72°C for 35 cycles. The PCR products were analyzed by Quantityone software (BioRad, USA). The change in mRNA expression was calculated by the 2^−ΔΔCT method.

Statistical analysis

SPSS 22.0 statistical analysis software was used to analyze the data. Data are expressed as mean ± standard deviation (SD). Independent samples were analyzed by χ² test and comparisons between groups were assessed by the t test. Survival rate was calculated with Kaplan-Meier analysis. Comparison of survival was made using the Mantel-Cox test. p<0.05 was considered statistically significant.

Results

B7H1 overexpression in urothelial bladder cancer tissue

The expression of B7-H1 protein was analyzed by immunohistochemistry (Figure 1). We found that the B7H1 expression was negative in normal bladder tissue. However, the rate of positive expression in urothelial bladder cancer tissue was 57.14% (56/98).

The correlation between B7H1 expression and clinicopathological parameters of UBC cases

We analyzed the correlation between the percentage of B7H1 expression and clinicopathological parameters (Table 2). The results showed that there was no association between B7H1-positive expression and either sex or age (p>0.05). Furthermore, B7H1 expression was not found to be associated with tumor metastasis, tumor size, or focus (p>0.05). According to the 2004 WHO criteria (low malignancy of urinary tract epithelial papilloma, low-grade Papillary urothelial carcinoma, high-grade papillary urothelial carcinoma), 98 specimens were classified as grade group and stage group. Interestingly, we found that B7H1 expression was increased in the high-grade group and in the stage group. In the grade groups, the percentages of B7H1 expression were 34.78%, 50.00%, and 86.20%, respectively. Similarly, the Ta-T1 group had 36% B7H1-positive expression and the T2-T4 group had 64.39% B7H1-positive expression. Tumor cases showed significantly different expression between B7H1 expression and grade group or stage group (p<0.05). Moreover, B7H1-positive expression was remarkably associated with tumor type (p=0.00). Twenty-two of 57 patients with primary tumors and 34 of 41 patients with tumor recurrence had B7H1-positive expression.

Association of B7H1 mRNA expression with patients’ clinical characteristics

The B7H1 mRNA levels were measured by RT-PCR (Figure 2A). The results were similar to the protein expression. The levels

![Figure 1. B7H1 protein expression in normal bladder tissue, UBC tissue, and positive tissue. Representative slides (*400) of B7H1 protein staining in normal bladder tissue (A), UBC tissue (B), and positive tissue (C).](image-url)
of B7H1 mRNA were obviously increased in UBC tissue compared to normal bladder tissue. Furthermore, we analyzed the relationship between B7H1 mRNA expression and clinical characteristics of UBC. A significant association was found between B7H1 mRNA expression and grade group or stage group (p<0.05, Figure 2B, 2C). In high-grade or stage group, the B7H1 mRNA levels were more pronounced. Moreover, the B7H1 mRNA expression in UBC patients had a significant correlation with tumor recurrence and metastasis (p<0.05, Figure 2D, 2E).

**Relationship of the expression of B7H1 to the rate of survival**

None of the included patients received radiation therapy or chemotherapy before or after the operation. Patients were

### Table 2. Connection between B7H1 expression and the clinicopathologic parameters of 98 UBC cases.

| Parameters | B7H1 positive expression (%) | p | Parameters | B7H1 positive expression (%) | p |
|------------|------------------------------|---|------------|------------------------------|---|
| Sex        |                              |   | Tumor type |                              |   |
| Female     | 59.09 (13/22)                | 0.517 | Primary    | 38.59 (22/57)                | 0.000 |
| Male       | 56.58 (43/76)                | 0.535 | Recurrence | 82.93 (34/41)                | 0.198 |
| Age (y)    |                              |   | Tumor size (diameter) |                              |   |
| <65        | 56.52 (26/46)                | 0.536 | Grade I    | 2.40 (20/82)                 | 0.013 |
| ≥65        | 57.69 (30/52)                | 0.536 | Grade II   | 2.20 (42/192)                | 0.465 |
| Tumor grade|                              |   | Grade III  | 2.00 (16/80)                 | 0.000 |
| I          | 34.78 (8/23)                 | 0.536 | Ta–T1      | 36.00 (9/25)                 | 0.000 |
| II         | 50.00 (23/46)                | 0.536 | T2–T4      | 64.39 (47/73)                | 0.000 |
| III        | 86.20 (25/29)                | 0.536 | Non-metastasis | 55.17 (47/87) | 0.198 |

**Figure 2. (A–E) The levels of B7H1 mRNA in specimens and the association between the B7H1 mRNA expression and clinical characteristics of UBC. * p<0.01, ** p<0.01.**
B7H1 was associated with the cancer grade accompanied by UBC tissue specimens. B7H1 is highly expressed in isolated expression [4]. Our study also shows that B7H1 is correlated with the immune escape process of UBC through up-regulation of B7H1 and activator of transcription 3 (JAK/STAT3) pathway and the phosphatidylinositol 3-kinase (PI3K)/AKT pathway in lung cancer [25]. These finding suggest the B7H1 is an important factor in tumor immunological escape.

followed up for 6 years from the day of the operation. During that time, 31 patients died due to recurrence of bladder cancer. The average survival time in patients with positive expression of B7H1 was 45 months, but it was 65 months in patients with negative expression of B7H1. The overall survival was significantly lower in patients with positive B7H1 expression than in those with negative B7H1 expression (62.50% vs. 90.48%, p=0.001).

Figure 3. Overall 6-year survival rate in 98 patients. The patients with positive B7H1 expression (n=56) had a poorer prognosis than those with negative B7H1 expression (n=42), P value was determined by the log-rank test (χ²=6.32, p=0.001).

Discussion

Urothelial bladder cancer is the second most common malignant GU tumor. There are 330 000 new cases each year worldwide and more than 50% of patients relapse [18], eventually evolving into invasive bladder cancer and developing into the muscular layer. The 5-year survival rate is far below 50% [19]. In the present study, we found that B7H1 expression in UBC was associated with tumor grade, stage, and type. Furthermore, the levels of B7H1 were associated with the postoperative prognosis. These results are consistent with previous studies [5,20].

The occurrence and development of bladder cancer is a multi-step and multi-stage process involving the activation of oncogenes, inactivation of tumor-suppressor genes, and many signaling pathways. It was reported that toll-like receptor (TLR4) expression was decreased in UBC patients [4], which could activate transcription by taking part in the MyD88/MEK/STAT1 signaling pathway [21]. TLR4 is a critical molecule in the regulation of immune response and it participates in the immune escape process of UBC through up-regulation of B7H1 expression [4]. Our study also shows that B7H1 is correlated with UBC tissue specimens. B7H1 is highly expressed in isolated cancer tissues [22,23]. In laryngeal cancer, the expression of B7H1 was associated with the cancer grade accompanied by high expressions of CD83+CD200+ cells [23]. In urothelial cancer, B7H1 was expressed at high levels on major populations of CD4+ and CD8+ tumor-infiltrating lymphocytes (TILs) [5]. T cells and several cytokines have been implicated as regulators of B7H1 expression in various cancers. Cytokines produced by the host are important in immune inhibition, including TNF-α and INF-γ [24, 25]. It was demonstrated that TNF-α and INF-γ induced PD-L1 expression by the Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway and the phosphatidylinositol 3-kinase (PI3K)/AKT pathway in lung cancer [25]. These finding suggest the B7H1 is an important factor in tumor immunological escape.

The results of our clinicopathological analyses support that B7H1 is a tumor-influencing factor in UBC. We observed that positive B7H1 expression was strongly associated with WHO grade, and higher grade was associated with higher expression. This result is similar to that reported by Nakaini [5]. In addition, we found there was a strong association between B7H1 positive expression and tumor recurrence or metastasis (Table 2, Figure 2). However, research has also found no association between B7H1-positive expression and clinic features such as stage and grade [20]. This result may be correlated with the number of participants or other factors in certain groups of patients. Nevertheless, it is clearly established that the positive expression of B7H1 predicts poor prognosis [5,20]. Our study also showed higher levels of B7H1 and lower survival in positive cases than in negative cases (Figure 3).

Research on the role of B7H1 expression in various types of cancer has been performed in many medical centers with many different sources of support [26–28]. Furthermore, many institutions have performed clinical studies on cancer treatment using B7H1 [29,30]. For example, in patients with metastatic melanoma treated with concurrent ipilimumab (anti-CTLA-4) and nivolumab (anti-B7H1), 17% of patients achieved a CR, with an overall survival (OS) rate for all patients of 79% at 2 years [31]. Many studies on bladder cancer have assessed the expression of B7H1 and its role in prognosis and targeted treatment. It is recommended that the expression of B7H1 should be assessed in bladder cancer patients after surgery, and it may be used as a target treatment in the future in bladder cancer [20,32,33].

Conclusions

In general, the expression of B7H1 is correlated with biological behavior in bladder urothelial cancer. Up-regulation of B7H1 can result in development of bladder cancer. B7H1 could be as a possible marker for urothelial carcinoma.
References:

1. Wang BH, Xie HY, Ma CG, et al. Expression of ARID1B is associated with poor outcomes and predicts the benefit from adjuvant chemotherapy in bladder urothelial carcinoma. J Cancer, 2017; 8: 3490-72.

2. Md Noh MSF, Abdul Aziz AF, Mohd Ghani KA et al. Giant intraductal bladder tumor. Am J Case Rep, 2017; 18: 212–16.

3. Wong JL, Woodward PJ, Manning MA et al: Neoplasms of the urinary bladder: Radiologic-pathologic correlation. Radiographics, 2006; 26: 553–80.

4. Wang YH, Cao YW, Yang XC et al: Effect of TLR4 and B7H1 on immune escape of urothelial bladder cancer and its clinical significance. Asian Pac J Cancer Pre, 2014; 15: 1321–26.

5. Nakashita J, Wada Y, Matsumoto K et al: Overexpression of B7H1 (PD1L) significantly associates with tumor grade and postoperative prognosis in human urothelial cancers. Cancer Immunol Immunother, 2007; 56: 1173–82.

6. Zirakzadeh AA, Kinn J, Krantz D et al: Doxorubicin enhances the capacity of B cells to activate T cells in urothelial urinary bladder cancer. Clin Immunol, 2017; 176: 63–70.

7. Topalian SL, Drake CG, Pardoll DM: Targeting the PD-1/B7-H1 (PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol, 2012; 24: 207–12.

8. Nishimura H, Nose M, Hirai H et al: Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity, 1999; 11: 141–51.

9. Xu P, Chen H, Chen YI et al: Expression of PD-1/PD-L1 in peripheral blood mononuclear cells in lung cancer patients and its biological significance. Zhonghua Zhenli Xue Za Zhi, 2013; 35: 910–13 [In Chinese].

10. Cunha LL, Marcello MA, Vassallo J, Ward LS: Differentiated thyroid carcinomas and their B7H1 shield. Future Oncol, 2013; 9: 1417–19.

11. Nishimura H, Nose M, Hira H et al: Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity, 1999; 11: 141–51.

12. Mao JT, Zhang L, Ma T et al: Anti-PD-L1 prolongs survival and triggers T cell but not humoral anti-tumor immune responses in a human MUC1-expressing preclinical ovarian cancer model. Cancer Immunol Immunother, 2015; 64: 1095–108.

13. Shi SJ, Wang L, Wang GD et al: B7-H1 expression is associated with poor prognosis in colorectal carcinoma and regulates the proliferation and invasion of HCT116 colorectal cancer cells. PLoS One, 2013; 8: e67012.

14. Kieffel S, Posch C, Barthel SR: Melanoma cell-intrinsic PD-1 receptor functions promote tumor growth. Cell, 2015; 162: 1242–56.

15. Qian Y, Deng J, Geng L et al: TLR4 signaling induces B7-H1 expression through MAPK pathways in bladder cancer cells. Cancer Invest, 2008; 26: 816–21.

16. Ni XY, Sui HX, Liu Y et al: TGF-β of lung cancer microenvironment upregulates B7H1 and GITRL expression in dendritic cells and is associated with regulatory T cell generation. Oncol Rep, 2012; 28: 615–21.

17. Ohigashi Y, Sho M, Yamada Y et al: Clinical significance of programmed death-1 ligand-1 and programmed death-1 ligand-2 expression in human esophageal cancer. Clin Cancer Res, 2005; 11: 2947–53.

18. Cheng T, Roth B, Choi W: Fibroblast growth factor receptors-1 and -3 play distinct roles in the regulation of bladder cancer growth and metastasis: implications for therapeutic targeting. PloS One, 2013; 8: e57284.

19. Tang L, Zirpoli GR, Guru K: Intake of cruciferous vegetables modifies bladder cancer survival. Cancer Epidemiol Biomarkers Prev, 2010; 19: 1806–11.

20. Xylinas E, Robinson BD, Kluh LA et al: Association of T-cell co-regulatory protein expression with clinical outcomes following radical cystectomy for urothelial carcinoma of the bladder. Eur Surg Oncol, 2014; 40: 121–27.

21. Liu J, Hamrouni A, Wolowiec D et al: Plasma cells from multiple myeloma patients express B7-H1 (PD-L1) and increase expression after stimulation with IFN-(gamma) and TLR ligands via a MyD88-, TRAF6-, and MEK-dependent pathway. Blood, 2007; 110: 296–304.

22. Du W, Zhu J, Chen Y et al: Variant SNPs at the microRNA complementary site in the B7-H1 3'-untranslated region increase the risk of non-small cell lung cancer. Mol Med Rep, 2017; 16: 2682–90.

23. Klatka J, Grywalska E, Klatka M et al: Expression of selected regulatory molecules on the CD83+ monocyte-derived dendritic cells generated from patients with laryngeal cancer and their clinical significance. Eur Arch Otorhinolaryngol, 2013; 270: 2683–93.

24. Martínez-Reza I, Díaz L, García-Becerra R: Preclinical and clinical aspects of TNF-α and its receptors TNFR1 and TNFR2 in breast cancer. J Biomed Sci, 2017; 24: 90.

25. Zhang X, Zeng Y, Qu Q et al: PD-L1 induced by INF-γ from tumor-associated macrophage via JAK/STAT3 and PI3K/AKT signaling pathways promotes progression of lung cancer. Int J Clin Oncol, 2017; 22: 1026–33.

26. Gordon MS, Hamid O, Powerley J et al: A phase I study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors. Abstract presented at the Annual Meeting of the American Association of Cancer Research 2013; Washington D.C., April 2013.

27. Taube JM, Anders RA, Young GD et al: Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. Sci Transl Med, 2012; 4: 127–37.

28. Topalian SL, Hodi FS, Brahmer JR et al: Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med, 2012; 366: 2443–54.

29. Bardoli AD, Afshar M, Viney R et al: The PD-1/PD-L1 axis in the pathogenesis of urothelial bladder cancer and evaluating its potential as a therapeutic target. Future Oncol, 2016; 12(5): 595–600.

30. Yu L, Wang Y, Shao S et al: B7-H1/PD-1 blockade therapy in urological malignancies: Current status and future prospects. Tumori, 2015; 101(5): 549–54.

31. Wolchok JD, Kluger H, Callahan MK et al: Nivolumab plus ipilimumab in advanced melanoma. N Eng J Med, 2013; 369: 122–33.

32. Powles T, Eder JP, Fine GD et al: MPDL3280A (anti-PD-L1) treatment leads to durable objective responses and improved survival in patients with non-small-cell lung cancer treated with platinum-based chemotherapy. J Clin Oncol, 2016; 34(2): 558–62.