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B7-H1 Blockade Enhanced the Function of Peripheral Blood Monocyte-Derived Dendritic Cells in Patients with Bladder Cancer

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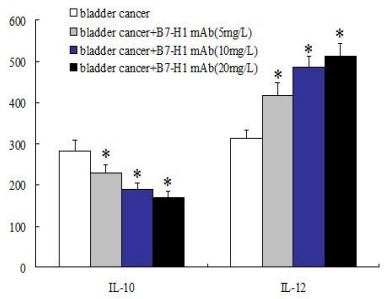
Abstract: **To investigate B7-H1 expressions on peripheral blood monocyte-derived dendritic cells (DCs) in patients with bladder cancer and the effect of B7-H1 blockade on DCs function. Monocyte-derived DCs (MoDCs) were generated from peripheral blood mononuclear cells, which were obtained from 30 patients with bladder cancer and 10 healthy donors as controls. The expressions of B7-H1 on MoDCs and PD-1 on $CD8^+$T cells were analyzed by flow cytometry. The effects of B7-H1 blockade on MoDCs capacity of stimulating T-cell proliferation and producing IL-10 and IL-12 were detected by mixed lymphocyte reaction and ELISA respectively. Compared with control, the capacity of MoDCs stimulating T-cell proliferation was significantly decreased in patients with bladder cancer. B7-H1 and PD-1 expressions were significantly up-regulated on MoDCs and $CD8^+$T cells respectively in patients with bladder cancer. Moreover, pre-incubation MoDCs with B7-H1 blocking mAb to block B7-H1 pathway resulted in a significant increase in T-cell stimulated proliferation and IL-12 secretion, while a significant decrease in IL-10 secretion. B7-H1 up-regulation may contribute to the functional deficiency of DCs in patients with bladder cancer, while B7-H1 blockade can restore and enhance DCs function in patients with bladder cancer.**



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1. Introduction

Dendritic cells (DCs) are the most potent antigen presenting cells (APCs), with the ability to present tumor antigens to naive or effector T lymphocytes. DCs play a vital role in the initiation, programming and regulation of tumor-specific immune responses. It has been shown that suppression of DC differentiation and function in cancer patients may contribute to inhibition of immune responses and cancer progression [1].

B7-H1 (also known as PD-L1), which normally expressed restrictedly in immune cells, is a recently discovered costimulatory molecule belonging to the B7 family. It can inhibit immune responses by binding to its receptor PD-1 on the surface of T lymphocytes and consequently inducing antigen-specific T-cell apoptosis or anergy. Recently, a variety of human tumor cell lines and freshly isolated human tumors have now been reported to aberrantly express B7-H1, including bladder cancer [2-5]. Moreover, it has been shown that B7-H1 might endow tumors with a mechanism to escape host immune destruction and early clinical trials have indicated effectiveness of B7-H1/PD-1 pathway blockade in several solid cancers. In 2014 Thomas Powles et al. reported that anti-B7-H1 antibody MPDL3280A had noteworthy activity in metastatic UBC, which was awarded a breakthrough for patients with bladder cancer after 30 years of a steady-state phase for treatments of this disease [6]. However, up to date the precise mechanism of action of B7-H1 in bladder cancer is not well understood. Recently, some studies found that tumor microenvironment could up-regulate B7-H1 expression on DCs and then down-regulate T-cell immunity by suppressing DCs' function. Our previous study also demonstrated that B7-H1 expression was significantly up-regulated on DCs from patients with bladder cancer [7]. All these results implied that B7-H1 may be a potential target for improvement of DC function.

In this study, we investigate B7-H1 expression on peripheral blood monocyte-derived DCs (MoDCs) in patients with bladder cancer and further test the effect of B7-H1 expression on the function of MoDCs. Here, we show that suppression of DCs function does exist in patients with bladder cancer. B7-H1 up-regulation may contribute to the functional deficiency of DCs in patients with bladder cancer, while blockade of B7-H1 can enhance DCs function in patients with bladder cancer.

2. Materials and Methods

2.1. Clinical samples

Peripheral blood was obtained from 10 healthy donors and 30 patients with bladder cancer who underwent surgical resection at the Affiliated Hospital, Qingdao University (Qingdao, China) between 2014 and 2015. Written informed consents were obtained and the study protocol was approved by the ethics committee of the Affiliated Hospital, Qingdao University. The patients included 24 males and 6 females with age ranging from 41 to 76 years (average 57 years). All the specimens of bladder cancer had been identified pathologically. Study exclusion criteria included: bladder infection before surgery, preoperative radiation therapy or chemotherapy, and preoperative biological therapy.

2.2. Preparation of peripheral blood-DCs

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-Hypaque density gradient centrifugation and then cultured in RPMI 1640 completed medium (containing 10% FCS). The cultures were supplemented with 1000 U/ml GM-CSF (Peprotech, USA) and 500 U/ml IL-4 (Peprotech, USA) on days 0, 2, and 4. TNF-α (1000 U/ml, Peprotech, USA) was used for maturation of iDCs on day 6. Blockade of B7-H1 on DCs was accomplished by incubating with monoclonal antibody (mAb) against B7-H1 (MIH1, 10 mg/L, eBioscience, USA).

2.3. FACS analysis for expressions of B7-H1 on DCs and PD-1 on $CD8^+$T cells

The staining of MoDCs and PBMCs followed the same basic procedure. MoDCs and PBMCs were collected and centrifuged for 3 minutes by 2000r / min rate. Then MoDCs were incubated with PE-labeled anti-B7-H1 Abs (eBioscience, USA), while PBMCs were incubated with PE-labeled anti-PD-1 Abs and PE-labeled anti-CD8 Abs (eBioscience, USA) at 4℃ for 30 minutes. After washing twice with PBS, the cells were analyzed immediately using flow cytometry (FACSC alibur, Becton Dickinson) and Cell Quest software (BD Biosciences).

2.4. Mixed lymphocyte reaction (MLR)

T cells, isolated from PBMCs with immunomagnetic microbeads, were cocultured with DCs from normal control, DCs from bladder cancer, or DCs from bladder cancer plus B7-H1 blockade in 96 well plates. Cultures were incubated for 2 days and then pulsed with 20μL/well of CCK-8 (Dojindo, Japan) for 4h. The absorbance of each well was measured with a microplate reader at 450 nm and each assay was repeated at least three times. Proliferation indices of T cells were measured by D value of experimental group/blank group.

2.5. ELISA for cytokine analysis

Supernatants were harvested immediately before the addition of CCK-8. IL-10 and IL-12 productions were detected by IL-10 ELISA kit and IL-12 ELISA kit respectively according to the manufacturer's instructions (R&D Systems, USA).

2.6. Statistical analysis

Statistical analysis was performed using SPSS 17.0. Data were obtained and enumerated by t-test. $P < 0.05$ was regarded as statistically significant.

3. Results

3.1. The capacity of stimulating T-cell proliferation in DCs from patients with bladder cancer

To investigate DC function contributing to tumor associated immune suppression in bladder cancer, we first examined the capacity of stimulating T-cell proliferation in DCs from patients with bladder cancer and normal controls. The capacity of stimulating T-cell proliferation was significantly decreased in DCs from patients with bladder cancer than in DCs from normal controls ($p<0.05$, Figure 1).

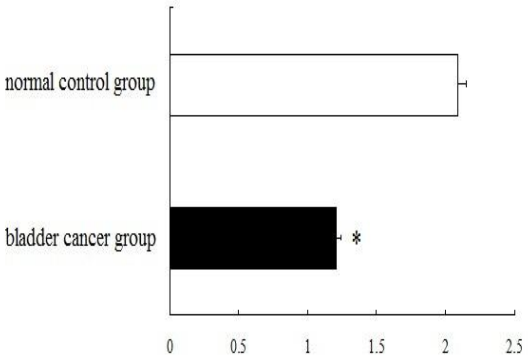

Figure 1. The capacities of MoDCs in normal controls and patients with bladder cancer to stimulate T-cell proliferation. * $p<0.05$ vs normal control group.

3.2. The expressions of B7-H1 on DCs and PD-1 on $CD8^+$ T cells from patients with bladder cancer

B7-H1 was weakly expressed on the surface of DCs from normal controls (9.69±2.35%). PD-1 was also weakly expressed on the surface of $CD8^+$ T cells from normal controls (4.56±1.77%). By contrast, B7-H1 and PD-1 expressions were both significantly up regulated on DCs (42.08±8.26%) and CD8+T cells (32.34±6.97) from patients with bladder cancer ($p<0.05$).

3.3. Blockade of B7-H1 enhances DCs function in patients with bladder cancer

To determine the role of B7-H1 in DC function and tumor immune escape, we blocked B7-H1/PD-1 pathway using a specific mAb of B7-H1 (MIH1). The treatment of DCs from patients with bladder cancer by B7-H1 blockade resulted in a significant increase in T-cell proliferation ($p<0.05$, Figure 2). Meanwhile, the effect of B7-H1 blockade on IL-10 and IL-12 production of DCs from patients with bladder cancer was also examined. Antibody against B7-H1 significantly increased IL-12 expression ($p<0.05$) and decreased IL-10 expression ($p<0.05$) in DC/T cultures (Figure 3).

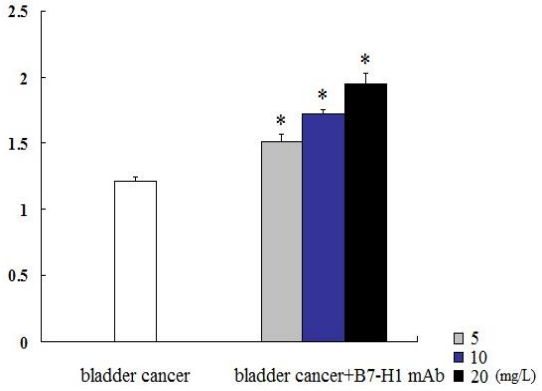

Figure 2. Effect of B7-H1 blockade on the capacity of MoDCs to stimulate T-cell proliferation in patients with bladder cancer. * $p<0.05$ vs bladder cancer group.

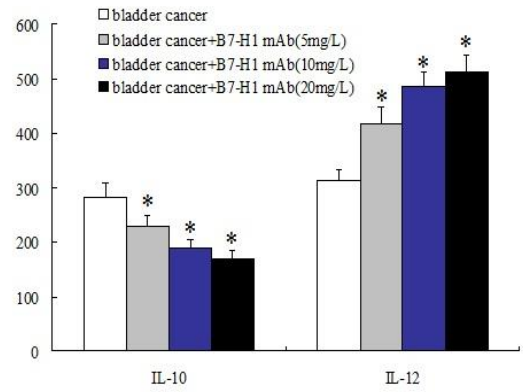

Figure 3. Effect of B7-H1 blockade on IL-10 and IL-12 secretions of MoDCs in patients with bladder cancer. * $p<0.05$ vs bladder cancer group.

4. Discussion

Bladder cancer has been characterized as an immunogenic cancer that contains large amounts of TILs and is sensitive to immunotherapy with BCG [8,9]. However, some studies have shown that patients with bladder cancer can manifest acquired tumor immune dysfunction, particularly affecting lymphocytes. Circulating T cells from patients with bladder cancer have been found to be unresponsive to polyclonal T-cell activation compared with healthy donor cells [10]. These evidences indicate that tumor immune escape may be a potentially important mechanism for pathogenesis and progression of bladder cancer. But the etiology of bladder cancer related immune dysfunction remains unclear.

T-cell mediated cytoimmunity is a main form of host antitumor immunity. Recognition and presentation of tumor antigen and activation of tumor specific T-cell is necessary for the start of tumor immune response. DCs, which are the most potent APCs, play an important role in the initiation, programming and regulation of tumor immune responses. Mature DCs express high levels of MHC and costimulatory molecules. Stimulatory and inhibitory signals presented by DCs during antigen presentation are integrated by the T cell and determine the final outcome of T cell activation. B7-H1 is a recently discovered T-cell suppressive costimulatory molecule that mainly expressed on the surface of activated monocytes and DCs. Tumor cell expression of B7-H1 can induce antigen-specific T-cell apoptosis or anergy and endow tumors with a mechanism to escape from host antitumor immunity [11]. It has been demonstrated that B7-H1 can play an inhibitory effect on DC function and blockade of B7-H1 on DCs results in enhanced T cell proliferation and cytokine production [12]. Recent studies have shown that tumor microenvironment factors could upregulate B7-H1 expression on DCs, which subsequently lead to impaired T-cell antitumor immunity [13]. It indicates that upregulation of B7-H1 on DCs may be a new mechanism by which human cancers evade immunity.

In this study we examined B7-H1 expressions on peripheral blood MoDCs in patients with bladder cancer and tested the effect of B7-H1 expression on the function of MoDCs. Here, we described that the capacity of MoDCs in patients with bladder cancer to stimulate T-cell proliferation was significantly decreased than that in normal controls. While B7-H1 and PD-1 expressions were significantly up-regulated on MoDCs and $CD8^+$T cells respectively in patients with bladder cancer. Furthermore, B7-H1 blockade could enhance the function of MoDCs in patients with bladder cancer by improving the capacity to stimulate T-cell proliferation, increasing IL-12 secretion and decreasing IL-10 secretion. It is well known that IL-12 is pivotal for enhancing Th1 type immunity and anti-tumor responses, while IL-10 facilitates Th2 type immunity which is undesirable and tumor-promoting. Therefore, our data suggest that suppression of DCs function does exist in patients with bladder cancer. B7-H1 up-regulation may contribute to the functional deficiency of DCs in patients with bladder cancer, while B7-H1 blockade can restore and enhance DCs function in patients with bladder cancer.

Because DCs play an important role in

tumor-specific immune responses, DC-based immunotherapeutic strategies have now developed from in vitro and murine studies to preclinical and clinical trials. However, there are still some challenges in generating DC products for therapy. It has been demonstrated that one of the major challenges is the immune tolerance induced by tumor cells [14]. Tumor cells can form an immunosuppressive environment by releasing factors such as B7-H1, which can inhibit DC function and downregulate tumor-specific immune responses. Therefore, our result implied that B7-H1 blockade may be a potential strategy in the immunotherapy of bladder cancer, not only by preventing T-cell apoptosis through B7-H1-expressing tumor cells, but also by inhibiting immunosuppression from B7-H1-expressing DCs.

Acknowledgements

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References

[1] Banchereau J, Steinman RM. Dendritic cells and the control of immunity[J]. Nature. 1998, 392(6673): 245-252.

[2] Moreno B H, Ribas A. Anti-programmed cell death protein-1/ligand-1 therapy in different cancers[J]. British journal of cancer, 2015, 112(9): 1421-1427.

[3] 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[J]. Clin. Cancer Res., 2004, 10:5094-5100.

[4] 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[J]. Neoplasia, 2006, 8:190-198.

[5] 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[J]. Cancer Res., 2006, 66:3381-3385.

[6] Powles T, Eder J P, Fine G D, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer[J]. Nature, 2014, 515(7528): 558-562.

[7] Wang S, Wang Y, Liu J, et al. Silencing B7-H1 enhances the anti-tumor effect of bladder cancer antigen-loaded dendritic cell vaccine in vitro[J]. OncoTargets and therapy, 2014, 7: 1389.

[8] Tsujihashi H, Matsuda H, Uejima S, et al. Immunoresponse of tissue infiltrating lymphocytes in bladder tumors[J]. J. Urol., 1989, 141:1467-1470.

[9] Patard JJ, Saint F, Velotti F, et al. Immune response following intravesical Bacillus Calmette-Guerin instillations in superficial bladder cancer: a review[J]. Urol. Res., 1998, 26:155-159.

[10] Loskog A, Ninalga C, Paul-Wetterberg G, et al. Human bladder carcinoma is dominated by T-regulatory cells and Th1 inhibitory cytokines[J]. J. Urol., 2007, 177:353-358.

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

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

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

[14] Bhargava A, Mishra D, Banerjee S, Mishra PK. Dendritic cell engineering for tumor immunotherapy: from biology to clinical translation[J]. Immunotherapy, 2012, 4(7): 703-718.

