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Original Article

Anti-prostate cancer effects of CTL cells induction by recombinant adenovirus mediated PSCA dendritic cells

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Abstract: Immunotherapy has shown evidence of efficacy in reducing the risk of death among primary and metastatic tumor patients. In this study, we develop an antitumor vaccination through cytotoxic T lymphocytes (CTL) induction by recombinant adenovirus (Ad) mediated prostate stem cell antigen (PSCA) dendritic cells (DC) for prostate cancer therapy. DC was stimulated to maturity through infecting with Ad carrying PSCA cDNA. After that, Ad^{PSCA}-DC utilized to induce CTL for prostate cancer therapy. The results revealed that Ad^{PSCA} infection improved DC maturation including the change of morphological and the expression of surface markers. Ad^{PSCA} infection up-regulated the secretion of interleukin-12, but down-regulated interleukin-10 secretion. CTL displayed high IFN-γ secretion level after Ad^{PSCA}-DC induced. In vitro, Ad^{PSCA}-DC-CTL markedly inhibited PC-3 cell proliferation related to DC-CTL at the effector: target ratio of 20:1. In vivo, Ad^{PSCA}-DC-CTL displayed strong prostate cancer cytotoxicity including inhibiting tumor formation, delaying tumor growth and improving mice survival rate. Pathological examination revealed that the numbers of infiltrating Ad^{PSCA}-DC-CTL within mice tumor tissues increased relative to DC-CTL. Through further in-depth investigation, the proposed Ad^{PSCA}-DC-CTL could serve as an effective anti-tumor immunity strategy against prostate cancer.

Keywords: Prostate cancer, cytotoxic T lymphocytes (CTL), prostate stem cell antigen (PSCA), dendritic cells (DC), adenovirus

Introduction

Prostate cancer that is one of the most common cancers in male's ranks second in cancer related deaths in Chinese men [1]. Date, the major options of prostate cancer treatment is surgery, chemotherapy and radiotherapy, but prostate cancer patients have not cured effectively [2]. Particularly, the curative treatment development for cancer patients, who has developed recurrent related disease because of conventional therapy failed or who has metastatic disease at diagnosis time, is failure. Lots of cancer therapy strategies are developed, such as photoacoustic therapy, photothermal therapy, immunotherapy, hormone ablation therapy et al [3, 4]. Hormone ablation therapy is a novel strategy and can offer palliation, but the majority of cancer patients eventually progress to refractory disease and further unresponsive to other cancer therapy [5]. Therefore, we need to develop novel and effective approaches for preventing and/or managing prostate cancer patients.

Immunotherapy is a promising method for eradicating tumor. Immunotherapy mechanism is that inducing systemic immunity to specific antigens that express on tumor cells but not normal prostate [6, 7]. Dendritic cells (DC), which are professional antigen presenting cells in the mammalian immune system, play pivotal roles in initiation and regulation of innate and adaptive immune responses [8]. The main function of DC is processing antigens captured in peripheral tissues, presenting them on the cell surface of T lymphocytes, and subsequently initiating T cell or B cell immunity [9]. In recent years, DC has been exploited extensively to develop DC-based immunotherapy to treat various puzzle diseases including virus and cancer. For example, ex vivo-generated DC combined with tumor-associated antigens could be as therapeutic vaccines against cancers such as metastatic melanoma, renal cell carcinoma, and B-cell lymphoma et al [10, 11]. Efficient delivery of antigens to DC, stimulate DC to maturation, and present tumor-specific antigens on

DC membranes are essential requirements for DC-based immunotherapy [12].

The recent study deepens our understanding of immune mechanism. There has a strong negative correlation relationship between CTL (cytotoxic T lymphocytes) number and cancer cell proliferation [13]. Regulating T-cell activation helps us develop better strategies for cancer therapy. Immunotherapy kernel is that inducing specific CTL responses depend on specific tumor-associated target antigens and effectively deliver antigens [13, 14]. Last several years, lots of prostate cancer specific antigens have been studied, such as prostate stem cell antigen (PSCA), prostate-specific antigen, prostatic acid phosphatase, prostate-specific membrane antigen and six-transmembrane epithelial antigen [15, 16]. These prostate-specific proteins could serve as ideal antigens that help CTL to identify and eliminate prostate cancer cells.

PSCA is an attractive therapeutic vaccine antigen because it is overexpression in prostate cancer, especially in metastatic tissues [17]. Significantly, it is limited expression in other organs and tissues [18]. PSCA having 123-amino acids is an ideal target antigen for this study. The PSCA amino acid sequence analysis shows that PSCA that belong to Ly-6/Thy-1 gene family is a membrane-bound protein [17]. Esophagus, stomach, bladder and prostate low express PSCA, but it overexpresses in 33% of primary prostate tumors and bone metastasis tissue [19]. PSCA expression level positive correlates with increasing tumor stage, grade et al [15]. Therefor PSCA is considered as an ideal target for cancer immunotherapy.

In this paper, we developed recombinant Ad encoding PSCA to infect DC for induction CTL and evaluated the cancer therapy efficacy of vaccine through PC-3 cell line and C57BL/6 mice prostate cancer experimental model. Our results showed that Ad^{PSCA}-DC-CTL displayed strong prostate cancer cytotoxicity including inhibiting PC-3 cell proliferation, tumor formation, delaying tumor growth and improving mice survival rate.

Materials and methods

Mouse and cancer cell line

Five weeks-old, pathogen-free, male BALB/c mice purchased from Animal experimental center of China Medical University. Institutional Animal Care and Use Committee guided the process of mice studies. PC-3 cells (human prostate cancer cells) cultured in high glucose DMEM (Dulbecco's modified Eagle medium) (Sigma, USA) added with 10% FBS (fetal bovine serum) (Thermo Fisher Scientific, USA), Nu-serum at 5% (BD Biosciences), dihydrotestosterone at 10 nmol/L and insulin at 5 mg/ml (Sigma, USA).

Construction of recombinant adenovirus

The shuttle vector pAdxsi Expression System (Calvino match Biological Technology Co. Ltd., China) was used for loading recombinant PSCA cDNA in Ad vector. PSCA cDNA (coding 95 amino acids) structured into the pShuttle-CMV to obtain pAdxsi-PSCA plasmid. pAdxsi-PSCA plasmid transacted into DC using Lipofectamine 2000 (Suzhou Baijishi Biological Technology Co. Ltd. China). The null vector (Ad^{null}) was applied as a control.

Generation of monocyte-derived DC

Peripheral blood mononuclear cells ($1\text{-}3\times10^{10}$) were collected from volunteers by leukapheresis, and then cultured in tissue culture flask (25 cm^2) adherence at a density of 1×10^7 cells/flask with 5 ml of RPMI-1640 medium (Sigma, USA) containing FBS (10%) and human serum albumin (1%) (Baxter, Deerfield, USA). After incubation 2 h (37°C, 5% CO_2), non-adherent cells were removed by rinsing with PBS (phosphate buffered saline). Then adherent cells were resuspended in clinical grade Cell-Gro DC medium (CellGenix, Freiburg, Germany) containing IL-4 (500 IU/mL) (CellGenix, Freiburg, Germany), 1000 IU/mL GM-CSF (CellGenix, Freiburg, Germany) for incubating 5 d. After that, DCs split into three aliquots. PBS, Ad-null and Ad^{PSCA} at MOI (multiplicity of infection) of 200 were added to DC culture. After 2 h incubation, FBS (10%) was added to RPMI 1640 medium, the medium consisted of recombinant GMCSF (1000 IU/ml), IL-4 at 500 IU/ml and 1000 IU/ml TNF-α. After 48 h of incubation, DC was harvested.

Preparation of PSCA-specifically stimulated CTL

Those DCs seeded into 24-well plates ($5\text{-}6\times10^4$ cells/well) after irradiated with 40 Gy, and then

we added non-adherent autologous peripheral blood lymphocytes (1-6×10^6 cells/well) from volunteers. Lymphocytes harvested after 7 days of co-culture, and then we seeded lymphocytes (5-6×10^5 cells/well). After that, we further stimulated those cells using irradiated DC (1-6×10^5 cells/well) for 7 days incubation. Then, we repeat the above process once again. Lymphocytes fed with IL-2 (50 U/ml) during co-culture. On the day 21 day, we purified CTL using DynabeadsH FlowCompTM CD8 Kit (Dynal biotech).

Flow cytometric analysis the marker of DC

Collected DC resuspended in cold PBS. After that, DC then incubated with APC (allophycocyanin)-conjugated mouse anti-human CD83 antibody (Abcam, USA), PE (phycoerythrin)-conjugated mouse anti-human CD80 antibody (abcam, USA), FITC-conjugated mouse anti-human CD86 antibody (abcam, USA), and Percp (peridinin-chlorophyll-protein complex)-conjugated mouse anti-human HLA-DR antibody (abcam, USA). cells (5-6×10^5) incubated with those fluorescence antibodies at 4°C (in dark, 40 min). Then those DC washed using PBS for three times, and then analyzed using FACScan (BD, USA).

Confocal fluorescence imaging

Ad^{PSCA}-DC was plated on confocal dish and cultured (37°C, 5% CO_2) for 12 h. APC-conjugated mouse anti-human CD83 antibody, Percp-conjugated mouse anti-human HLA-DR antibody and DAPI (Beijing Biodee Diagnostic Technology Co. Ltd. China) were co-incubated with the cells for 1 h. After PBS washed, cell image performed using laser scanning confocal microscopy (ZEISS LSM 510 META, Germany). APC was excited at 752 nm and recorded at 720-750 nm; Percp was excited at 488 nm and recorded above 677 nm, DAPI was excited at 340 nm and recorded above 488 nm.

Detection of the level of IL-12 and IL-10 in the Ad^{PSCA}-DC supernatant

Optimum MOI200 added to DC after five days of culture, and the corresponding virus added as well. DC transfections divided into 3 groups: Ad^{PSMA}-DC, Ad^{null}-DC and DC control groups. After six days of transfection, TNF-α (Shanghai Puxin Biological Technology Co. Ltd. China) (1 000 U/mL) was added to stimulate DC to mature; after 6 days of transfection, ELISA was employed to detect the level of IL-12 and IL-10 in the cell culture supernatant of each group.

Detection of IFN-γ

DCs, Ad^{null}-DC and Ad^{PSCA}-DC cells supplemented with fresh medium and IL-2 to induce CTL cells every three days. CTL cell concentration was adjusted to 1×10^6 cells/ml. Mixed cultures were prepared for three groups consisting of DC-CTL, Ad^{null}-DC-CTL and Ad^{PSCA}-DC-CTL on 96-well plates. The IFN-γ on DC-CTL, Ad^{null}-DC-CTL and Ad^{PSCA}-DC-CTL were measured using an ELISPOT assay.

Detection cytotoxicity effects of Ad^{PSCA}-DC-CTL

PC-3 prostate cancer cells collected, and then seeded into 48-well plates (5-6×10^4 cells/well) in 2 ml culture medium. CTL (1-6×10^6 cells) was added into 48-well plates at 20:1 of E:T after 4 h static cultivation. Cell mixture was washed using cold PBS after 12 h incubation. Those cells stained with propidium iodide (PI) and annexin V-FITC (Invitrogen, USA), and then using flow cytometry (BD Bioscience, USA) analyzed data. Fluorescent emission of FITC was 515-545 nm and excitation of FITC was at 488 nm, fluorescent emission of DNA-PI complexes was 564-606 nm and excitation was at 488 nm. Compensation was used wherever necessary.

Immunization and tumor challenge

Nude BALB/c mice (male) were intravenous injection of 1×10^6 DC-CTL, Ad^{null}-DC-CTL and Ad^{PSCA}-DC-CTL. After one week, mice were subcutaneous injection of 2×10^6 PC-3 cells at vertebral flank. 40 days following last vaccination, tumor volume was calculated using formula $V = (L \times W^2)/2$, where W was shortest dimension and L was longest dimension. For therapeutic effectiveness detection, 2×10^6 PC-3 cells were subcutaneous injection into the vertebral flank. When mice tumor volume developed to 100 mm^3, (1×10^6) DC-CTL (1×10^6), Ad^{null}-DC-CTL (1×10^6) and Ad^{PSCA}-DC-CTL (1×10^6) were intravenous injected at once a week. Three weeks after injection, mouse weight, tumor sizes and medium survival times were recorded.

Histological staining

Tumors collected from the euthanized PC-3 cells tumor bearing BALB/c male mice after

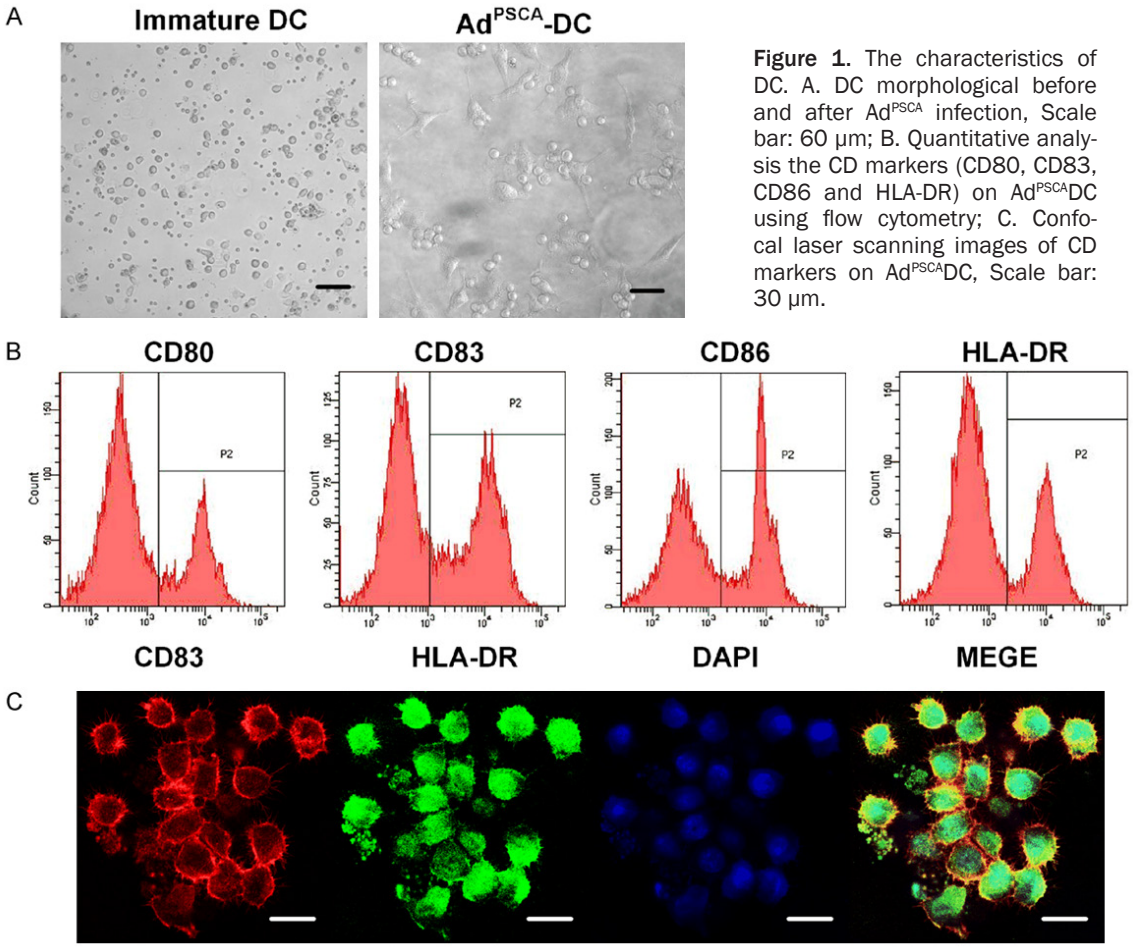

Figure 1. The characteristics of DC. A. DC morphological before and after Ad^{PSCA} infection, Scale bar: 60 μm; B. Quantitative analysis the CD markers (CD80, CD83, CD86 and HLA-DR) on Ad^{PSCA}DC using flow cytometry; C. Confocal laser scanning images of CD markers on Ad^{PSCA}DC, Scale bar: 30 μm.

immunotherapy. Tumor tissue was fixed in a formaldehyde (4%) solution (Aladdin, China) for at least 48 h at room temperature. H&E staining (BBC Biochemical, USA) and immunohistochemical staining were performed and then observed with a BX41 bright field microscopy (Olympus, Japan).

Statistical analysis

Statistical analysis was conducted with SPSS 19.0 software. Results were expressed as mean ± SD. Student's t test was used to assess differences. $P < 0.05$ represented statistically significant.

Results

Characteristics of DC

DC was isolated from human peripheral blood and activated through infecting with Ad^{PSCA} in vitro. DC morphological under a microscope (**Figure 1A**) displayed typical modifications from small, unequal size and round to large and cell aggregation. After that, we then detected the expression of surface molecule on Ad^{PSCA}-DC by FACS. The FACS data in **Figure 1B** showed that the percentage of surface markers CD80, CD83, CD86 and HLA-DR was 39.6%, 55.2%, 62.4% and 67.8%, respectively. Those surface molecules on Ad^{PSCA}-DC were further studied using confocal laser scanning microscope (CLSM). Confocal images (**Figure 1C**) showed CD83 and HLA-DR appeared obviously on Ad^{PSCA}-DC surface. Those results illustrated that DCs induced successfully to maturity by Ad^{PSCA}.

Immune factors expression level of DC and CTL

IL-10 and IL-12 are significant immunoregulatory cytokine, which have important biological function. We detected the expression levels of IL-10 and IL-12 of DC by ELISA. As shown in **Figure 2A** and **2B**, IL-12 expression level in DC,

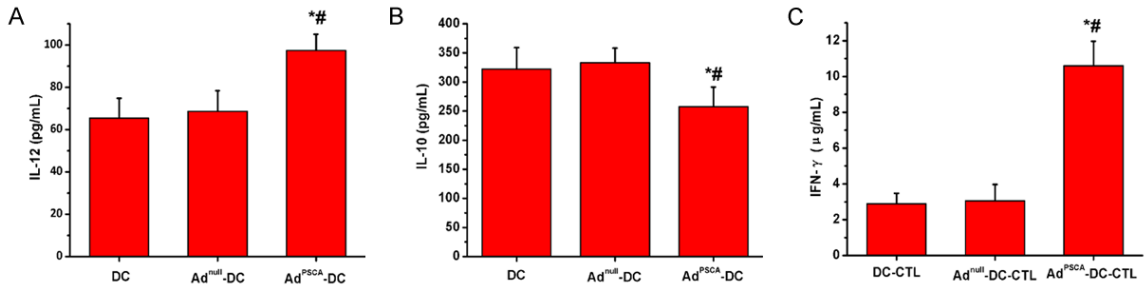

Figure 2. The immune factors expression level of DC and CTL. A. ELISA detection of IL-12 expression level of DC, Ad^{null}-DC and Ad^{PSCA}-DC; B. ELISA detection of IL-10 expression level of DC, Ad^{null}-DC and Ad^{PSCA}-DC; C. ELISPOT assay the IFN-γ level of CTL, Ad^{null}-DC-CTL and Ad^{PSCA}-DC-CTL. Compared with DC, *P < 0.05; Compared with Ad^{null}-DC, #P < 0.05.

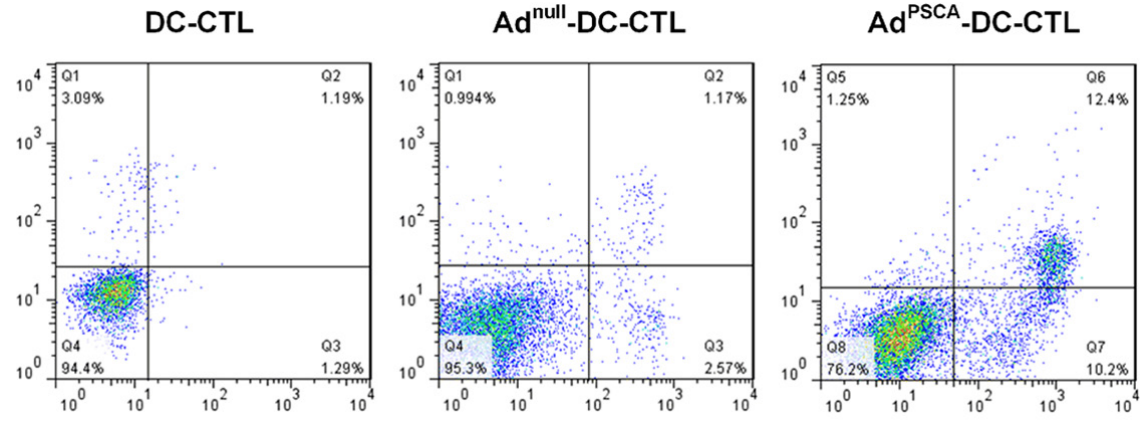

Figure 3. Annexin V-FITC/propidium iodide (PI) double staining analysis of CTLs cytotoxic against PC-3 cells.

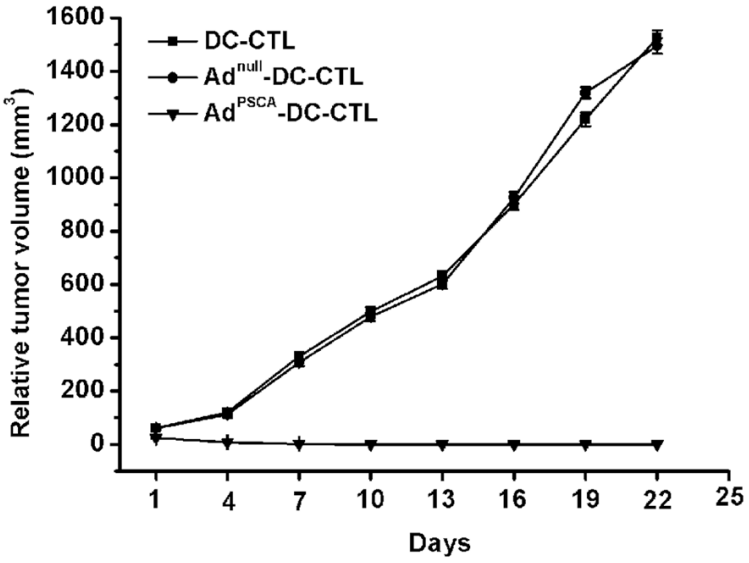

Figure 4. Anti-tumor protection of DCs, Ad-null-DCs and Ad-PSCA-DCs.

Ad^{null}-DC and Ad^{PSCA}-DC followed by 65.49 ± 9.31, 68.62 ± 9.83 and 97.37 ± 7.76 (pg/ml) and the IL-10 expression level in DC, Ad^{null}-DC and Ad^{PSCA}-DC followed by 322.46 ± 36.88, 333.13 ± 2 5.19 and 257.92 ± 33.63 (pg/ml); Compared with DC, IL-12 in Ad^{PSCA}-DC secretion increased 1.49 times, but the secretion of IL-10 in Ad^{PSCA}-DC reduced a fifth. Meanwhile, either IL-12 or IL-10 was not significant difference between DCs and Ad^{null}-DC. Ad^{PSCA} improved the secretion of IL-12 and inhibited the IL-10 secretion. It suggested that immunological reaction of our study was inclined to the Th1 response.

IFN-γ plays a role in tumor cell growth and apoptosis. IFN-γ in CTL with different stimulation was detected using ELISPOT assay. As showed in **Figure 2C**, IFN-γ level of CTL stimulated with

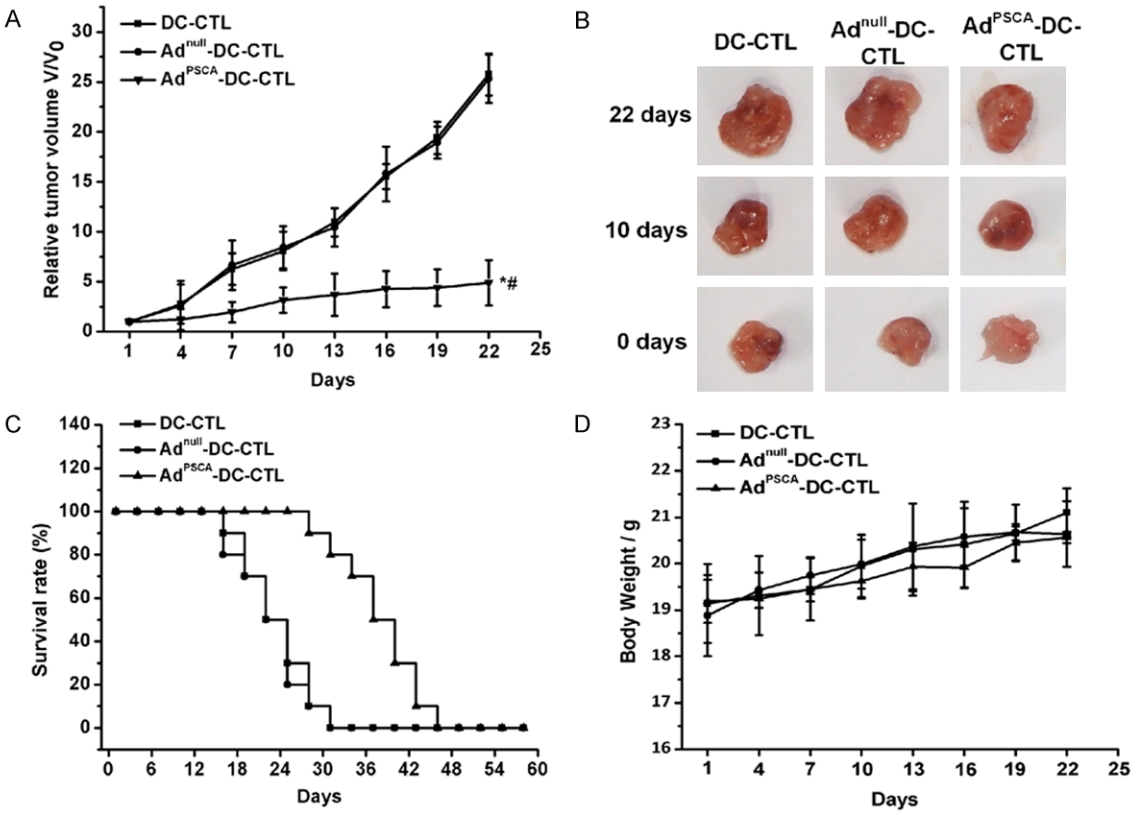

Figure 5. Tumor treatment effects of Ad^{PSCA}-DC-CTL in vivo. A. The quantitative analysis of tumor size after various treatments; B. The quantitative analysis of body weight after various treatments; C. The medium survival times of the mice after various treatments; D. Representative photos of tumor after various treatments.

DC, Ad^{null}-DC and Ad^{PSCA}-DC were 1.88 ± 0.58, 3.05 ± 0.92 and 10.59 ± 1.38 (μg/L), respectively. Ad^{PSCA}-DC-CTL expressed the highest level of IFN-γ compared with other CTL groups.

Ad^{PSCA}-DC stimulated CTL cytotoxic activity in vitro

To quantitatively evaluate the tumor therapeutic efficacy of Ad^{PSCA}-DC, cell viabilities of PC-3 cells with different treatment protocols were evaluated using annexin V-FITC/PI double staining analysis. The result (**Figure 3**) illustrated that DC-CTLs, Ad-null-DCs-CD8+CTL and Ad^{PSCA}-DC-CTL caused 5.6%, 3.7% and 23.8% of PC-3 cells to lysis, respectively, at 20:1 ratio of E:T. Ad^{PSCA}-DC-CTL displayed enhanced killing activity among three different kinds of CTL. The killing efficiency of Ad^{PSCA}-DC-CTL was 4.25 fold compared with DC-CTL and 6.4 fold compared with Ad^{null}-DC-CTL. It declared that Ad^{PSCA}-DC-CTL had enhanced cytotoxicity and provided an effective alternative cancer treatment modality.

Evaluation of therapeutic effect of Ad^{PSCA}-DC-CTL in vivo

To evaluate therapeutic effect of Ad^{PSCA}-DC-CTL in mice model, firstly, we investigated the function of Ad^{PSCA}-DC-CTL to suppress tumor formation in male nude BALB/c mice. Before subcutaneous injecting PC-3 tumor cells (4×10^6/mice), mice were injection with DC-CTL, Ad^{null}-DC-CTL and Ad^{PSCA}-DC-CTL, respectively. After 22 days, there was not a tumor located in mice on Ad^{PSCA}-DC-CTL group. However, tumor developed in DC-CTL group and Ad^{null}-DC-CTL group, the size of tumor was up to 1525.74 ± 28.1 mm^3 and 1495.31 ± 28.41 mm^3, respectively (**Figure 4**). Those results declare that Ad^{PSCA}-DC-CTL can effectively and markedly prevent the formation of tumors

We further researched the anticancer activities of Ad^{PSCA}-DC-CTL in tumor-bearing mice. When tumor volumes grew up to 100 mm^3, prostate tumor bearing mice treated with DC-CTL, Ad^{null}-DC-CTL and Ad^{PSCA}-DC-CTL, respectively. Follow-

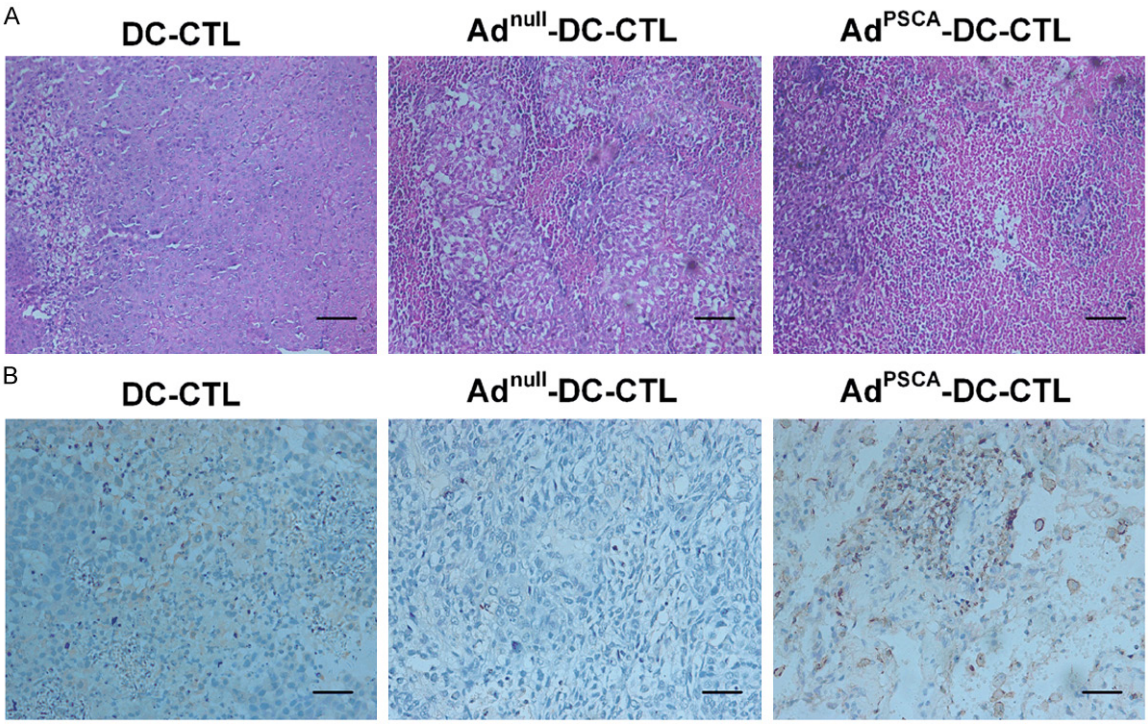

Figure 6. Pathological examination analysis of tumor tissue. A. H&E stained images of tumor collected from the tumor bearing mice after various treatments, Scale bar: 90 μm; B. Immunohistochemistry stained images of tumor tissues isolated from immune mice, Scale bar: 60 μm.

ing the therapy, we monitored the tumor volumes and body weights, periodically. Those quantitative analysis data (**Figure 5A**) showed that DC-CTL group and Ad^{null}-DC-CTL group had a similar tumor size that rapidly grew up to 25 folds by day 22. However, mice treated with Ad^{PSCA}-DC-CTL for 22 days had a small tumor growth up to 5 folds, it significantly lower than others. Representative photos of tumor after various treatments showed the same result (**Figure 5B**). The medium survival times of mice that treated with DC-CTLs and Ad^{null}-DC-CTL, respectively, were 22 and 22 days, respectively. Significantly, mice treated with Ad^{PSCA}-DC-CTL survived up to 37 days (**Figure 5C**). One of the immunotherapy side effects is the loss of body weight. In our study, we measured mice body weights of different groups for 22 d, the result in **Figure 5D** showed that mice body weights of different groups were stable. To further assess the toxicity, the pathological examination was performed by hematoxylin and eosin (H&E) staining. The result (**Figure 6**) indicates that, compared with DC-CTL group, tumor tissue of Ad^{PSCA}-DC-CTL group showed severe edema and vacuolation cells, while unnumbered Ad^{PSCA}-DC-CTL appear on the tumor tissues, suggesting a reasonable therapy magic for the application. It declared that our immunotherapy strategy had low toxicity. We further examined the footprint of Ad^{PSCA}-DC-CTL by immunohistochemistry for making sure whether Ad^{PSCA}-DC-CTL could effectively permeate into tumor microenvironment. As show as in **Figure 6B**, immunohistochemistry stained images revealed vast of infiltrating Ad^{PSCA}-DC-CTL within tumor tissues relative to DC-CTL. These data declared that the strong expansion of tumor-specific Ad^{PSCA}-DC-CTL correlates to the inhibition of tumor growth by immunization.

Discussion

To develop antitumor vaccine, DC was collected from the peripheral blood of human, and further infected with recombinant Ad encoding PSCA. Ad is chosen as the main candidate for delivering target genes. It can integrate cDNA with genome of host cell by integrase for persistently expressing PSCA protein [20, 21]. It is beneficial to DC maturation and activation. Importantly, Ad is a safe vector that doesn't

induce side-effects including cancer, immunological rejection and inflammation [22, 23]. DC matured always with CD80, DC83, CD86, and HLA-DR high protein expression [24]. At molecular levels, compared with DC and Ad^{null}-DC, we found that Ad^{PSCA}-DC expressed highly level of cell surface marker. The cell surface marker is conductive to the interaction of DCs and native T lymphocyte, it help native T lymphocyte differentiation and maturation. Morphologically, the Ad^{PSCA}-DC showed typical size and shape changes, transfection with Ad could also enhance DC maturation. The mechanism may be that Ad^{PSCA} entry into DC and/or translocation to DCs nuclei. Ad capsid protein penton activated NF-κB to up-regulate a lot of protein expression such as immune response, TNF-α, an immune response protein, improves DC maturation through an autocrine pathway [25, 26]. DC is the strongest professional antigen-presenting cell. PSCA disassembled to peptide in DC and then connected with MHC-I before transporting to cellular membrane for T cell antigen receptor recognition [27].

Activated DC can secrete various cytokines, such as IL-12 and IL-10 [28]. In our study, Ad^{PSCA}-DC possessed the character of the highest expression level of IL-12 and the lowest expression level of IL-10. High expression IL-12 could initiate protective innate and adaptive immune responses against cancer cell [29]. In immunological reaction, IL-12 can be beneficial to develop Th1 type reaction that induces the IFN-γ expression for killing tumor cells [30]. However, IL-10 as an inhibitory factor initials Th1 type reaction and T lymphocyte. Ad^{PSCA} infection inhibits IL-10 secretion that up regulates the expression level of IFN-γ, TNF-α and GM-CSF [31-33]. Inhibit IL-10 secretion enhances antigen-presenting ability and inhibits immunity escape of carcinoma [34].

Antigen-specific CTL is a promising immunotherapeutic cell against cancers. T-cell receptor (TCR) of native T lymphocyte recognizes specific major histocompatibility complex (MHC)-antigen complexes along with co-stimulatory molecules [35]. It initiates related signaling pathway and promotes native T lymphocyte differentiate into CD8+CTL and CD4+CTL. Simultaneously, the proliferation capacity of CTL enhanced. In this study, we found that CTL was induced successfully by Ad^{PSCA} infected DC. Cell-mediated immunity based on CD8+CTLs plays a primary role in cancer therapy. Nevertheless, the mutual recognition of TCR on native T lymphocytes and tumor antigen presented by DC is under the influence of cancer cells killing efficiency [36]. PSCA up-regulates in some cancers surface including prostate cancer, bladder cancer and pancreatic cancers [17, 37]. The PSCA expression level positively correlated with prostate cancer metastasis, advanced clinical stage and malignant progression of premalignant prostate lesions [38]. Therefore, PSCA was chose as an ideal target antigen. Ad^{PSCA}-DC-CTL had strong cytotoxic activity against PC-3 cell which highly expressed PSCA. IFN-γ expression also confirmed that CTL stimulated by Ad^{PSCA}-DC had remarkable cytotoxic activity. IFN-γ can activate NK cells, and it is a marker for activating Th1 cells [39]. IFN-γ activates JAK-STAT pathway through IFN-γ receptor for killing cancer cells [40]. Ad^{PSCA}-DC-CTL was useful and could further develop for prostate cancer immunotherapy in vivo.

We further perform in vivo animal experiment to verify our current data. Ad^{PSCA}-DC-CTL vaccination suppressed exogenous PC-3 prostate cancer cell to format tumor. Importantly, Ad^{PSCA}-DC-CTL delayed the development of xenograft tumor. Immunohistochemical staining showed that lots of Ad^{PSCA}-DC-CTL appeared in tumor tissue, it was beneficial to damage tumor cell. There are two ways for Ad^{PSCA}-DC-CTL to kill tumor cells. One is the secretion of perforins and granzymes [41]. In the presence of Ca^{2+}, perforin can be inserted into tumor cell membranes, and polymerization forming tubular structure to destroy the structure of the target cell membranes, while granzymes as a kind of serine esterases, enter the cytoplasm to directly activate protease in cytoplasm leading to the cell apoptosis [42, 43]. On the other hand, high level FasL on CTL and Fas on the surface of tumor cells mutual recognized to make the tumor cell programmed cell death [44, 45].

Conclusion

DCs that infected with recombinant Ad encoding PSCA specifically activated cytotoxic T lymphocytes against prostate cancer. Ad^{PSCA}-DC-CTL markedly inhibited PC-3 cell proliferation. In vivo, Ad^{PSCA}-DC-CTL displayed strong prostate cancer cytotoxicity including inhibiting tumor formation, delaying tumor growth and improv-

ing mice survival rate. It provides a novel strategy for immunotherapy of prostate cancers.

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Disclosure of conflict of interest

None.

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References

[1] Grossmann M, Cheung AS, Zajac JD. Androgens and prostate cancer; pathogenesis and deprivation therapy. Best Pract Res Clin Endocrinol Metab 2013; 27: 603-616.

[2] Zamboglou N, Tselis N, Baltas D, Buhleier T, Martin T, Milickovic N, Papaioannou S, Ackermann H, Tunn UW. High-dose-rate interstitial brachytherapy as monotherapy for clinically localized prostate cancer: treatment evolution and mature results. Int J Radiat Oncol Biol Phys 2013; 85: 672-678.

[3] Zang Y, Wei Y, Shi Y, Chen Q, Xing D. Chemo/photoacoustic dual therapy with mrna-triggered DOX release and photoinduced shockwave based on a DNA-Gold nanoplatform. Small 2016; 12: 756-769.

[4] Slovin S, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal J, Scher HI, Chin K, Gagnier P, McHenry MB, Beer TM. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013; 24: 1813-1821.

[5] Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, Wong YN, Hahn N, Kohli M, Cooney MM, Dreicer R, Vogelzang NJ, Picus J, Shevrin D, Hussain M, Garcia JA, DiPaola RS. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. New Engl J Med 2015; 373: 737-746.

[6] Tran E, Turcotte S, Gros A, Robbins PF, Lu YC, Dudley ME, Wunderlich JR, Somerville RP, Hogan K, Hinrichs CS, Parkhurst MR, Yang JC, Rosenberg SA. Cancer immunotherapy based on mutation-specific CD4+ T cells in a patient with epithelial cancer. Science 2014; 344: 641-645.

[7] Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science 2015; 348: 69-74.

[8] Idoyaga J, Fiorese C, Zbytnuik L, Lubkin A, Miller J, Malissen B, Mucida D, Merad M, Steinman RM. Specialized role of migratory dendritic cells in peripheral tolerance induction. J Clin Invest 2013; 123: 844-854.

[9] Plantinga M, Guilliams M, Vanheerswynghels M, Deswarte K, Branco-Madeira F, Toussaint W, Vanhoutte L, Neyt K, Killeen N, Malissen B, Hammad H, Lambrecht BN. Conventional and monocyte-derived CD11b+ dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. Immunity 2013; 38: 322-335.

[10] Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, Thatcher N, Wagstaff J, Zielinski C, Faulkner I, Mellstedt H. Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol 2014; 11: 509-524.

[11] Palucka K, Banchereau J. Dendritic-cell-based therapeutic cancer vaccines. Immunity 2013; 39: 38-48.

[12] Hacohen N, Fritsch EF, Carter TA, Lander ES, Wu CJ. Getting personal with neoantigen-based therapeutic cancer vaccines. Cancer Immunol Res 2013; 1: 11-15.

[13] Coulie PG, Van den Eynde BJ, van der Bruggen P, Boon T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat Rev Cancer 2014; 14: 135-146.

[14] Vesely MD, Schreiber RD. Cancer immunoediting: antigens, mechanisms, and implications to cancer immunotherapy. Ann NY Acad Sci 2013; 1284: 1-5.

[15] Abate-Daga D, Lagisetty KH, Tran E, Zheng Z, Gattinoni L, Yu Z, Burns WR, Miermont AM, Teper Y, Rudloff U, Restifo NP, Feldman SA, Rosenberg SA, Morgan RA. A novel chimeric antigen receptor against prostate stem cell antigen mediates tumor destruction in a humanized mouse model of pancreatic cancer. Hum Gene Ther 2014; 25: 1003-1012.

[16] Brazhnik K, Sokolova Z, Baryshnikova M, Bilan R, Efimov A, Nabiev I, Sukhanova A. Quantum dot-based lab-on-a-bead system for multiplexed detection of free and total prostate-specific antigens in clinical human serum samples. Nanomedicine 2015; 11: 1065-1075.

[17] Taeb J, Asgari M, Abolhasani M, Farajollahi MM, Madjd Z. Expression of prostate stem cell antigen (PSCA) in prostate cancer: a tissue microarray study of Iranian patients. Pathol Res Pract 2014; 210: 18-23.

[18] Kwon OJ, Zhang L, Xin L. Stem cell Antigen-1 identifies a distinct androgen-independent mu-

rine prostatic luminal cell lineage with bipotent potential. Stem Cells 2016; 34: 191-202.
[19] Wang L, Sang Y, Tang J, Zhang RH, Luo D, Chen M, Deng WG, Kang T. Down-regulation of prostate stem cell antigen (PSCA) by Slug promotes metastasis in nasopharyngeal carcinoma. J Pathol 2015; 237: 411-422.
[20] Tan WG, Jin HT, West EE, Penaloza-MacMaster P, Wieland A, Zilliox MJ, McElrath MJ, Barouch DH, Ahmed R. Comparative analysis of simian immunodeficiency virus gag-specific effector and memory CD8+ T cells induced by different adenovirus vectors. J Virol 2013; 87: 1359-1372.
[21] Lion T. Adenovirus infections in immunocompetent and immunocompromised patients. Clin Microbiol Rev 2014; 27: 441-462.
[22] Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, Hensley L, Ammendola V, Abbate A, Grazioli F, Foulds KE, Cheng C, Wang L, Donaldson MM, Colloca S, Folgori A, Roederer M, Nabel GJ, Mascola J, Nicosia A, Cortese R, Koup RA, Sullivan NJ. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nat Med 2014; 20: 1126-1129.
[23] Westphal M, Ylä-Herttuala S, Martin J, Warnke P, Menei P, Eckland D, Kinley J, Kay R, Ram Z; ASPECT Study Group. Adenovirus-mediated gene therapy with sitimagene ceradenovec followed by intravenous ganciclovir for patients with operable high-grade glioma (ASPECT): a randomised, open-label, phase 3 trial. Lancet Oncol 2013; 14: 823-833.
[24] Weber M, Hauschild R, Schwarz J, Moussion C, de Vries I, Legler DF, Luther SA, Bollenbach T, Sixt M. Interstitial dendritic cell guidance by haptotactic chemokine gradients. Science 2013; 339: 328-332.
[25] Cui H, Zhang W, Hu W, Liu K, Wang T, Ma N, Liu X, Liu Y, Jiang Y. Recombinant mammaglobin A adenovirus-infected dendritic cells induce mammaglobin A-specific CD8+ cytotoxic T lymphocytes against breast cancer cells in vitro. PLoS One 2013; 8: e63055.
[26] Blengio F, Raggi F, Pierobon D, Cappello P, Eva A, Giovarelli M, Varesio L, Bosco MC. The hypoxic environment reprograms the cytokine/chemokine expression profile of human mature dendritic cells. Immunobiology 2013; 218: 76-89.
[27] Hudecek M, Lupo-Stanghellini MT, Kosasih PL, Sommermeyer D, Jensen MC, Rader C, Riddell SR. Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. Clin Cancer Res 2013; 19: 3153-3164.
[28] Ali OA, Tayalia P, Shvartsman D, Lewin S, Mooney DJ. Inflammatory cytokines presented from polymer matrices differentially generate and activate DCs in situ. Adv Funct Mater 2013; 23: 4621-4628.
[29] McNab FW, Ewbank J, Howes A, Moreira-Teixeira L, Martirosyan A, Ghilardi N, Saraiva M, O'Garra A. Type I IFN induces IL-10 production in an IL-27-independent manner and blocks responsiveness to IFN-γ for production of IL-12 and bacterial killing in mycobacterium tuberculosis-infected macrophages. J Immunol 2014; 193: 3600-3612.
[30] Tillack C, Ehmann LM, Friedrich M, Laubender RP, Papay P, Vogelsang H, Stallhofer J, Beigel F, Bedynek A, Wetzke M, Maier H, Koburger M, Wagner J, Glas J, Diegelmann J, Koglin S, Dombrowski Y, Schauber J, Wollenberg A, Brand S. Anti-TNF antibody-induced psoriasiform skin lesions in patients with inflammatory bowel disease are characterised by interferon-γ-expressing Th1 cells and IL-17A/IL-22-expressing Th17 cells and respond to anti-IL-12/IL-23 antibody treatment. Gut 2014; 63: 567-577.
[31] Curtale G, Mirolo M, Renzi TA, Rossato M, Bazzoni F, Locati M. Negative regulation of Toll-like receptor 4 signaling by IL-10-dependent microRNA-146b. Proc Natl Acad Sci U S A 2013; 110: 11499-11504.
[32] Ruffell B, Chang-Strachan D, Chan V, Rosenbusch A, Ho CM, Pryer N, Daniel D, Hwang ES, Rugo HS, Coussens LM. Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. Cancer Cell 2014; 26: 623-637.
[33] Gezen-Ak D, Dursun E, Hanağası H, Bilgiç B, Lohman E, Araz ÖS, Atasoy IL, Alaylıoğlu M, Önal B, Gürvit H, Yılmazer S. BDNF, TNFα, HSP90, CFH, and IL-10 serum levels in patients with early or late onset Alzheimer's disease or mild cognitive impairment. J Alzheimer's Dis 2013; 37: 185-195.
[34] von Lanzenauer S, Wolk K, Höflich C, Kunz S, Grünberg B, Döcke W, Reineke U, Asadullah K, Sterry W, Volk HD, Sabat R. Interleukin-10 receptor-1 expression in monocyte-derived antigen-presenting cell populations: dendritic cells partially escape from IL-10's inhibitory mechanisms. Genes Immun 2015; 16: 8-14.
[35] Pierce BG, Weng Z. A flexible docking approach for prediction of T cell receptor-peptide-MHC complexes. Protein Sci 2013; 22: 35-46.
[36] Rangarajan S, Mariuzza RA. T cell receptor bias for MHC: co-evolution or co-receptors? Cell Mol Life Sci 2014; 71: 3059-3068.
[37] Zhao Y, Gui Z, Liao S, Gao F, Ge Y, Jia R. Prostate stem cell antigen rs2294008 (C>T) polymorphism and bladder cancer risk: a meta-analysis based on cases and controls. Genet Mol Res 2014; 13: 5534-5540.

[38] Kloss CC, Condomines M, Cartellieri M, Bachmann M, Sadelain M. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nat Biotech 2013; 31: 71-75.
[39] Tu TC, Brown NK, Kim TJ, Wroblewska J, Yang X, Guo X, Lee SH, Kumar V, Lee KM, Fu YX. CD160 is essential for NK-mediated IFN-γ production. J Exp Med 2015; 212: 415-429.
[40] Blahoianu MA, Rahimi AA, Kozlowski M, Angel JB, Kumar A. IFN-γ-induced IL-27 and IL-27p28 expression are differentially regulated through JNK MAPK and PI3K pathways independent of Jak/STAT in human monocytic cells. Immunobiology 2014; 219: 1-8.
[41] Voskoboinik I, Whisstock JC, Trapani JA. Perforin and granzymes: function, dysfunction and human pathology. Nat Rev Immunol 2015; 15: 388-400.
[42] Dotiwala F, Mulik S, Polidoro RB, Ansara JA, Burleigh BA, Walch M, Gazzinelli RT, Lieberman J. Killer lymphocytes use granulysin, perforin and granzymes to kill intracellular parasites. Nat Med 2016; 22: 210-216.
[43] Naito T, Baba T, Takeda K, Sasaki S, Nakamoto Y, Mukaida N. High-dose cyclophosphamide induces specific tumor immunity with concomitant recruitment of LAMP1/CD107a-expressing CD4-positive T cells into tumor sites. Cancer Lett 2015; 366: 93-99.
[44] Künkele A, Johnson AJ, Rolczynski LS, Chang CA, Hoglund V, Kelly-Spratt KS, Jensen MC. Functional tuning of CARs reveals signaling threshold above which CD8+ CTL Antitumor potency is attenuated due to cell Fas-FasL-Dependent AICD. Cancer Immunol Res 2015; 3: 368-379.
[45] Miller MR, Mandell JB, Beatty KM, Harvey SA, Rizzo MJ, Previte DM, Thorne SH, McKenna KC. Splenectomy promotes indirect elimination of intraocular tumors by CD8+ T cells that is associated with IFNγ-and Fas/FasL-dependent activation of intratumoral macrophages. Cancer Immunol Res 2014; 2: 1175-1185.