



Anti-prostate cancer effects of CTL cell induction *in vitro* by recombinant adenovirus mediated PSMA/4-1BBL dendritic cells: an immunotherapy study

C.-G. Sui, D. Wu, F.-D. Meng, M.-H. Yang and Y.-H. Jiang

Department of Tumor Biotherapy and Cancer Research,
First Affiliated Hospital of China Medical University, Shenyang,
Liaoning, China

Corresponding author: C.-G. Sui
E-mail: suichengguang_1@163.com

Genet. Mol. Res. 14 (2): 7208-7217 (2015)
Received September 19, 2014
Accepted March 12, 2015
Published June 29, 2015
DOI <http://dx.doi.org/10.4238/2015.June.29.14>

ABSTRACT. This study aimed to examine anti-prostate cancer immune response induced by dendritic cells (DCs) transduced with PSMA/4-1BBL recombinant adenoviruses *in vitro*. Ad-PSMA, Ad-4-1BBL, and Ad-GFP were transfected into DCs derived from peripheral blood of healthy volunteers. Ad-PSMA/4-1BBL-DC, Ad-PSMA-DC, Ad-4-1BBL-DC, Ad-GFP-DC, and normal-DC, PSMA and 4-1BBL protein levels in DCs were detected by western blot. IL-12, IFN- γ and IL-10 were measured by ELISA. Mixed lymphocyte reaction and the cytotoxicity of each group targeted to LNCap, Du145, and 22RV prostate cancer cells were determined by CCK-8 assay. PSMA and 4-1BBL protein could express on DC successfully, the IL-12 supernatant content (134.29 ± 2.22 pg) was higher than others ($P < 0.05$). The ability to stimulate autologous T lymphocyte proliferation in the co-transfection group was higher than others ($P < 0.05$). When the DCs were co-cultured with CTLs, the PSMA/4-1BBL-DC-CTL

group showed the highest content of IFN- γ (1176.10 ± 14.37 pg/ 5×10^6 cells), but the lowest IL-10 content (75.14 ± 2.01 pg/ 5×10^6 cells) ($P < 0.05$), and the strongest anti-tumor effect when the effector to target ratio was 40:1, along with a higher killing ratio of LNCap cells than others ($P < 0.05$). Overall, Mature DCs transfected with Ad-PSMA/4-1BBL not only showed high secretion of IL-12, but also induced CTLs to stimulate and enhance the killing effect of PSMA specific effector cells to PSMA positively expressing prostate cancer cells. Furthermore, the DCs infected with two kinds of tumor-associated antigens would induce more effective tumor-specific CTL induction.

Key words: Dendritic cell; HPSMA gene; Cytotoxic T lymphocyte; 4-1BBL gene

INTRODUCTION

In Western countries, prostate cancer is the second most common malignancy (Jemal et al., 2008). In recent years, the morbidity of prostate cancer has also shown a rising trend (Gu, 2000). The therapies for prostate cancer include surgery, radiotherapy, chemotherapy, and endocrine therapy, and whereas these treatments have a therapeutic effect, the curative effect is either not satisfactory, or the treatments have serious adverse reactions, especially for hormone-independent and post-operative recurrence and metastasis prostate cancer patients, spurring the search for new therapies. Dendritic cell (DC) vaccine therapy has become a promising area of research for prostate cancer immunotherapy. Prostate-specific membrane antigen (PSMA) is highly specifically expressed in prostate cancer tissues making it one of the best options for targeted therapy (Perner et al., 2007). 4-1BBL, which is expressed on the surface of antigen-presenting cells, is a costimulatory molecule that plays an important role in immune response, immunomodulation, anti-tumor and anti-viral infection defense, and in transplant rejection, and has become an important area of immunologic research (Wang, 2003; Dawicki and Watts, 2004). In this study, we have constructed a new prostate cancer DC vaccine by co-transfecting DCs with recombinant adenovirus carrying *PSMA* and *4-1BBL* genes in order to observe whether the ability of DC prostate-specific membrane antigen presentation and of T cell excitation is enhanced, or, on the other hand, whether the generation of CTLs is induced. These experiments will allow the observation of the therapeutic effects of this approach on prostate cancer, laying the foundation for further application to *in vivo* experiments, with the anticipation that this study will provide a new paradigm for DC-based prostate cancer immunotherapy.

MATERIAL AND METHODS

Materials and reagents

The Ad-PSMA-GFP, Ad-4-1BBL-GFP, and Ad-GFP vectors were constructed and maintained in our laboratory; peripheral blood mononuclear cells were obtained from healthy volunteers; and the three kinds of prostate cancer cell lines were maintained by our laboratory. RPMI1640 and fetal calf serum were purchased from Gibco (Gaithersburg, MD, USA), and recombinant human GM-CSF, recombinant human IL-4, and recombinant human TNF- α

were purchased from Xiamen Amoytop Biotech Company (Xiamen, China). Phosphate buffered saline (PBS) and lymphocyte separation medium were purchased from Tian Jin Haoyang Biological Manufacture Co., Ltd. (Tianjin, China), total matriptase, proteinase inhibitor, and CCK8 assay kits were purchased from Beyotime Biotech Company (Shanghai, China). Prestained protein markers were purchased from MBI company, (Lithuania), rabbit anti human and 4-1BBL monoclonal antibodies were purchased from Epitomics (Burlingame, CA, USA), and horseradish peroxidase-conjugated goat anti rabbit secondary antibody was purchased from Santa Cruz Biotechnology (Dallas, Texas, USA). Rabbit anti human β -actin polyclonal antibody and ECL substrate were purchased from CWBIO company (Beijing, China); L-12, IFN- γ and IL-10 ELISA assay kits were purchased from Dakewe Biotech Company (Shenzhen, China).

Experimental methods

Induction and cultivation DC and CTL cells from peripheral blood in vitro

Peripheral blood mononuclear cells from healthy volunteers were separated and enriched using Haemonetics MCS+ collection system (HAEMONETICS, The Blood Manufacture Co., Ltd., USA), bone marrow mononuclear cells were isolated by density gradient centrifugation with a Ficoll-Hypaque solution. Cells were washed twice with PBS, and cell counts and viability were detected by Trypan blue staining. Cells were resuspended in RPMI1640 culture medium containing 10% serum with adjustment of the cell concentration to 2×10^6 /mL, and inoculated into 25 cm² culture flasks. Cells were allowed to adhere at 37°C, 5% CO₂ for two h. Non-adherent cells were transferred to a new culture flask for *in vitro* CTL cell induction. Adherent cells were cultured with RPMI1640 medium containing 10% fetal calf serum, GM-CSF 100 ng/mL and IL-4 50 ng/mL. The medium was changed every three days, and cytokines were added, induced for 6 days, and DCs were stimulate to mature by addition of 1000 U/mL TNF- α ; mature DCs were harvested after 7 to 8 days.

Detection of PSMA and 4-1BBL protein levels on DCs by western blot

After 5 days growth the semi-adherent immature DCs were collected, and made into a single cell suspension. The experiment was divided into four groups and corresponding amounts of virus was added according to the transfection group: MOI200, Ad-PSMA/4-1BBL co-transfection, Ad-PSMA-DC, Ad-4-1BBL-DC, and Ad-GFP-DC groups. Cells were cultured in a 37°C, 5% CO₂ incubator for 48 h, lysed by using radioimmunoprecipitation assay (RIPA) buffer with addition of protease inhibitor, and centrifuged at 12,000 rpm for 5 min at 4°C. The supernatant was transferred to a new Eppendorf tube, and stored at -20°C. The sample was subjected to electrophoresis using a 10% separating gel with a 5% stacking gel. After electrophoresis the protein was transferred to a polyvinylidene fluoride (PVDF) membrane by the wet transfer method, blocked with 5% nonfat milk powder at room temperature for 2 h, and incubated at 4°C overnight following addition of rabbit anti-human PSMA monoclonal antibody at 1:2000 dilution, or rabbit anti-human 4-1BBL monoclonal antibody at 1:1000 dilution. The next day, the membrane was washed with Tris buffered saline/Tween 20 (TBST) three times for 10 min each, and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies (1:2500) at room temperature for 1 h. Membranes were washed again with TBST three times for 10 min each, and developed with ECL substrate in the dark room.

Detection of the level of IL-12 in the DC supernatant vaccine culture in each group

Optimum MOI200 was added to DCs after five days of culture, and the corresponding virus was added as well. DC transfections were divided into five groups: Ad-PSMA/4-1BBL-DC, Ad-PSMA-DC, Ad-4-1BBL-DC, Ad-GFP-DC, and DC control groups. After six days of transfection, TNF- α (1000 U/mL) was added to stimulate DCs to mature; after 6 days of transfection, ELISA was employed to detect the level of IL-12 in the cell culture supernatant of each group.

CCK-8 assay detection of proliferation of T lymphopoiesis stimulated by DCs from different transfection groups

After 5 days growth the semi-adherent immature DCs were collected, the corresponding virus was added according to the optimum MOI200, and cells were divided into 5 groups: Ad-PSMA/4-1BBL-DC, Ad-PSMA-DC, Ad-4-1BBL-DC, Ad-GFP-DC, and an un-transfected DC control group. Cells were cultured in a 37°C, 5% CO₂ incubator for 48 h for preparation of cell stimulation. Mitomycin C was added to a final concentration of 10 μ g/mL before use, inoculated at 37°C for 30 min, washed three times with 1640 incomplete culture, and the cell concentration adjusted to 1 x 10⁶ cells/mL. For responding cell preparation, mononuclear cells were suspended in 1640 culture containing 10% fetal calf serum with addition of IL-2 (1000 U/mL), and supplemented with fresh medium and IL-2 to induce CTL cells every three days; cell concentration was adjusted to 1 x 10⁶ cells/mL. Mixed cultures were prepared for the five groups consisting of stimulating (DC) cells and T cells in ratios of 1:10, 1:20, 1:40 on 96-well plates; 20 μ L WST-8 were added to each well, and the OD value of each well was measured after 4 h reaction with an enzyme immunoassay analyzer according to CCK-8 manufacture instruction, to determine the influence of different transfection group DC vaccines on T cell proliferation. This method was also used to determine the optimum cell ratio of DC and T cells.

Determination of the level of IFN- γ , IL-10 in the co-culture supernatants of DC vaccines and CTLs by ELISA

According to the results of the mixed lymphocyte reaction described above, after 96 h co-culture of DC vaccine and CTL, the culture supernatant (the concentration of CTLs was approximately 2x10⁶ cells/mL) was collected by 1200 rpm centrifugation at 4°C for 5 min, and stored at -20°C. The secretory levels of IFN- γ and IL-10 were determined and analyzed for each group using the IFN- γ , IL-10 ELISA assay kit according to kit instructions.

CCK-8 assay detection of the cytotoxicity of different transfection DC-CTL cell groups on LNCap, Du145, and 22RV prostate cancer cell lines

The stimulating cells (DC vaccine of each group) and effector cells (CTL cells) were prepared as described above, co-cultured at the optimum co-culture ratio, with adjustment of the concentration of suspended cells to 2x10⁶ cells/mL with complete medium. Cells were incubated at 37°C, 5% CO₂ for 96 h, and 1 x 10⁴ cells per well of the prostate cancer cells LNCap, Du14, and 22RV were inoculated respectively to the 96-well plates. After 24 h adherence, different ratios (10:1, 20:1, 40:1) of DC-CTL cells (five groups) were added along with CTL cells as a negative control, cultured another 24 h, followed by addition of 20 μ L CCK8 per

well, and incubation at 37°C for 4 h. The OD value of each well was measured, and the killing ratio calculated using the formula: killing ratio (%) = $[1 - (\text{experimental group OD} - \text{effector cells alone group OD}) / \text{target cell alone group OD}] \times 100\%$.

Statistical analysis

Data were analyzed using the SPSS 16.0 statistical software (SPSS, Chicago, IL, USA) and reported as (means \pm SD). Comparisons were performed using one-way analysis of variance among groups. $P < 0.05$ or $P < 0.01$ was considered to be statistically significant.

RESULTS

Detection of PSMA and 4-1BBL protein levels on DCs by western blot

The collected DC protein levels of the Ad-PSMA/4-1BBL co-transfection, Ad-PSMA, Ad-4-1BBL, and Ad-GF transfection groups were determined by western blot. The results showed that PSMA could be effectively expressed in DCs in the Ad-PSMA transfection and co-transfection groups. The bands in the Ad-4-1BBL transfection and co-transfection groups suggested that the 4-1BBL protein could be effectively expressed, and neither protein was expressed in the Ad-GFP transfection group (Figure 1).

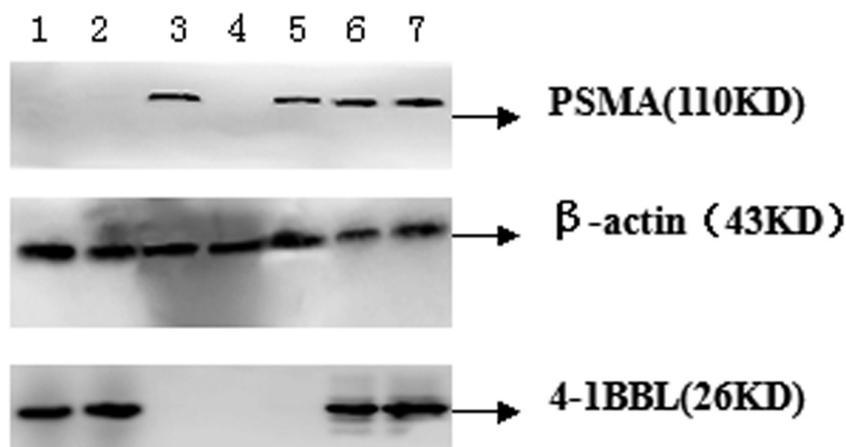


Figure 1. Identification of expression of human PSMA and 4-1BBL on dendritic cells by western blot. (Lanes 1 and 2 = from Ad-GFP-4-1BBL transfected dendritic cells; lanes 3 and 5 = from Ad-GFP-PSMA transfected dendritic cells; lane 4 = from empty Ad-GFP transfected dendritic cells; lanes 6 and 7 = from cotransfected dendritic cells).

Determination of the IL-12 content in the transfection and non-transfection DC culture supernatants

After 48 h transfection each group of mature DC vaccine and non-transfection culture supernatants were collected, and the IL-12 content determined by ELISA (Figure 2). The content of IL-12 in each DC culture transfection group was higher than in the non-transfection group ($P < 0.05$); furthermore, the content of the IL-12 Ad-PSMA/4-1BBL-DC co-transfection

group (134.29 ± 2.22 pg/ 2×10^6 cells) was higher than any single-transfection group (134.29 ± 2.22) vs (79.51 ± 1.60), (70.33 ± 1.13), (69.67 ± 1.43), (28.88 ± 2.97) pg ($P < 0.05$). These results suggested that transfection of DCs with PSMA and 4-1BBL mediated by recombinant adenovirus can greatly increase their IL-12 secretory ability, especially in the Ad-PSMA/4-1BBL-DC group.

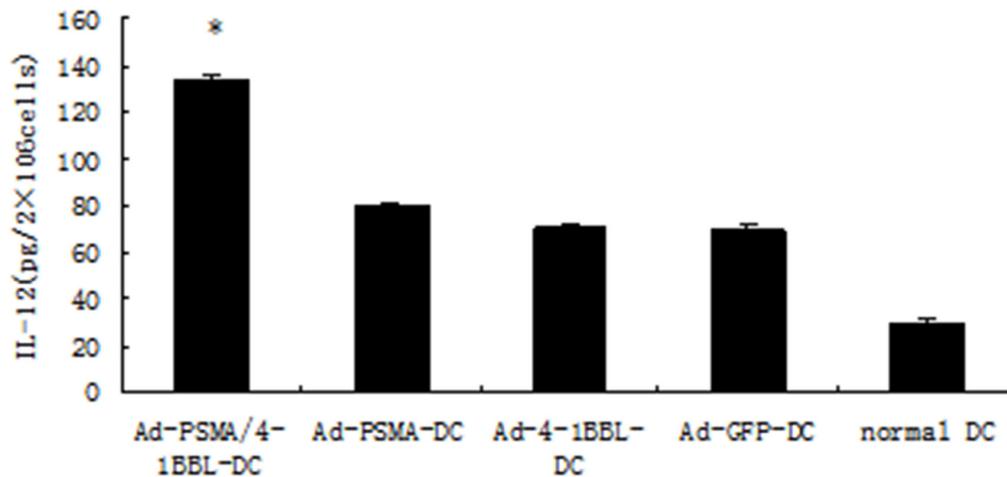


Figure 2. IL-12 production of each kind of DC vaccine by ELISA (* $P < 0.05$ vs ctrl).

Influence of recombinant adenovirus infection on MLR

According to the results of the CCK-8 assay (Figure 3), the OD value of each well, and the stimulating index (SI) = experimental group D value/(responding cell control group D value + stimulating cells control group D value), that each transfected DC group appeared to be able to stimulate autologous T lymphocyte proliferation. At the same ratio of DC:T cells, the ability to stimulate autologous T lymphocyte proliferation in the co-transfection group was remarkably higher than that of the other transfection groups and the non-transfection group ($P < 0.05$); no other obvious differences were found between the other single-transfection groups. The optimum co-culture ratio of DC and T cell was found to be 1:10.

Detection of IFN- γ and IL-10 levels in each culture supernatant of CTLs induced by the DC vaccine

The results of ELISA (Figures 4 and 5) showed that the levels of IFN- γ in CTL cell culture supernatants under the stimulation of transfected DCs are all higher than those of the DC-CTL control group and the single CTL control group ($P < 0.05$); however, the content of IL-10 was lower than that in the DC-CTL and single CTL control groups ($P < 0.05$, except for the GFP-DC-CTL and DC-CTL groups). In particular, the level of IFN- γ PSMA/4-1BBL-DC-CIK group showed the highest content, reaching 1176.10 ± 14.37 pg/ 5×10^6 cells, but the level of IL-10 was the lowest (75.14 ± 2.01 pg/ 5×10^6 cells) compared to other groups ($P < 0.05$).

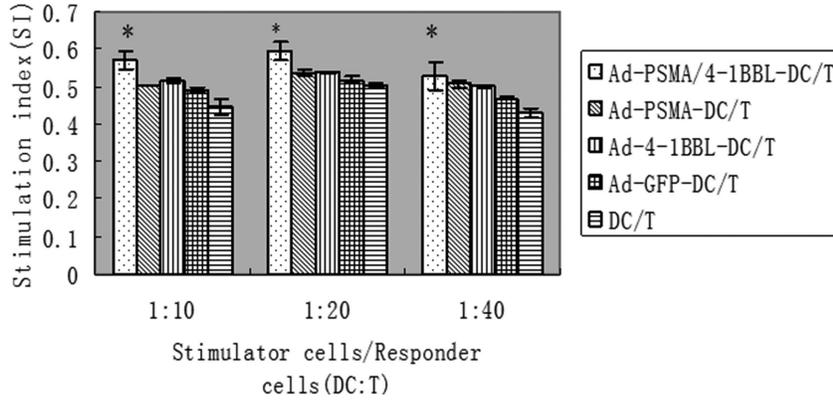


Figure 3. Effect of recombinant adenovirus infection on proliferation of T lymphocytes *P < 0.05 vs Ad-PSMA-DC/T, Ad-4-1BBL-DC/T, Ad-GFP-DC/T, DCs/T, respectively.

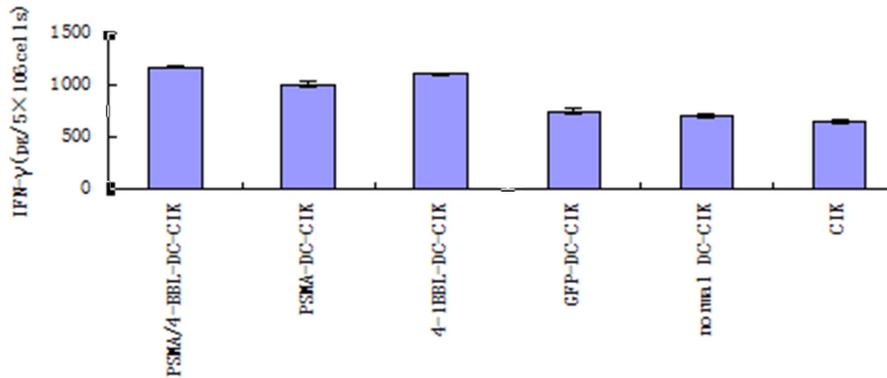


Figure 4. IFN-γ production of CTL cells cocultured with each kind of DC vaccine by ELISA (*P < 0.05 vs ctrl).

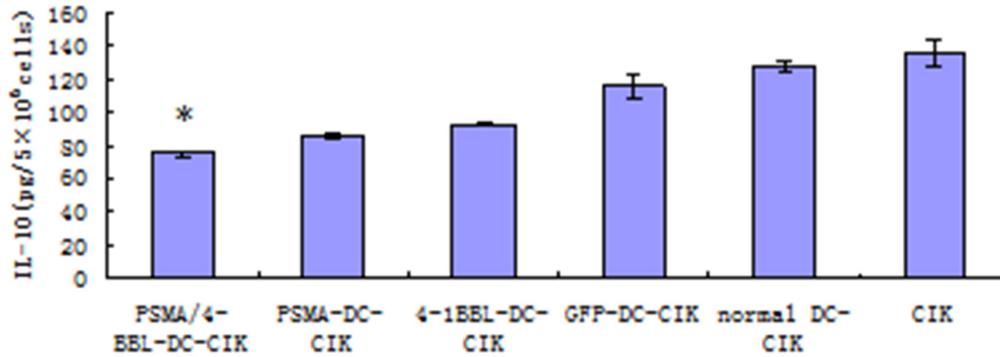


Figure 5. IL-10 production of CTL cells cocultured with each kind of DC vaccine by ELISA (*P < 0.01 vs ctrl).

Cytotoxicity effect of effector cells (CTLs) to target cells (LNCaps, Du145s, and 22RVs)

The killing ratio was calculated according to the OD value of each well as determined by the CKK8 assay: (%) = [1 - (Experimental group OD value - effector cells group alone OD value)/target cells group alone OD value] x 100%. The killing ratios of CTLs induced by virus infection of the three kinds of prostate cancer cells were all remarkably higher than the control DC stimulation and CTL control groups. The strongest killing effect was observed when the effector and target ratio was 40:1. At the same effector and target ratio, the killing effect of Ad-PSMA/4-1BBL-DC-CTL, Ad-PSMA-DC-CTL, and Ad-4-1BBL-DC-CTL groups to LNCap, that highly express PSMA, were remarkably higher than of the other two kinds of prostate cancer cell lines (Du145 and 22RV) ($P < 0.05$). In addition, compared to Ad-PSMA-DC-CTLs, Ad-4-1BBL-DC-CTLs, and other groups, the killing ratio for the three kinds of prostate cancer cells of the Ad-PSMA/4-1BBL-DC-CTL group showed significant differences, whereas there was no significant difference between the Ad-GFP-DC-CTL, DC-CTL control, and CTL control groups ($P > 0.05$). However, the d-GFP-DC-CTL, DC-CTL control, and CTL control groups showed significant differences ($P < 0.05$), as shown in Table 1. These results suggested that more effectively induction of tumor specific CTLs can occur when DCs are transfected with two kinds of tumor-associated antigens, PSMA/4-1BBL, than with either singly, which can differentially enhance the killing effect of prostate cancer cells (LNCap) positively expressing PSMA.

Table 1. Killing ability of effector cells (CTL) to three kinds of prostate cancer cell.

| Target cell | E:T | Ad-PSMA/4-1BBL-DC-CTL | Ad-PSMA-DC-CTL | Ad-4-1BBL-DC-CTL | Ad-GFP-DC-CTL | Normal DC-CTL | CTL |
|-------------|------|----------------------------|----------------------------|----------------------------|---------------|---------------|---------------|
| LNCap | 10:1 | 75.24 ± 0.66 ^{▲*} | 72.18 ± 0.43 ^{▲*} | 69.52 ± 1.63 ^{▲*} | 64.28 ± 1.63 | 63.96 ± 1.47* | 37.90 ± 1.39* |
| | 20:1 | 81.78 ± 1.55 ^{▲*} | 80.87 ± 1.50 ^{▲*} | 80.56 ± 1.43 ^{▲*} | 76.92 ± 0.92* | 74.51 ± 0.85* | 51.08 ± 0.86* |
| | 40:1 | 92.56 ± 0.56 ^{▲*} | 89.76 ± 0.44 [▲] | 89.70 ± 1.21 ^{▲*} | 80.97 ± 1.12 | 78.75 ± 0.99* | 62.35 ± 0.97* |
| DU145 | 10:1 | 68.71 ± 0.63 [▲] | 66.61 ± 0.95 [▲] | 65.24 ± 2.04 [▲] | 63.22 ± 1.86 | 60.83 ± 1.03 | 35.81 ± 0.76 |
| | 20:1 | 78.23 ± 1.68 ^{▲*} | 74.49 ± 1.10 [▲] | 73.23 ± 0.33 [▲] | 66.96 ± 0.82 | 64.73 ± 1.68 | 40.90 ± 1.10 |
| | 40:1 | 85.52 ± 0.82 ^{▲*} | 80.21 ± 1.20 [▲] | 79.83 ± 1.07 [▲] | 76.66 ± 0.37 | 73.52 ± 1.29 | 51.84 ± 2.24 |
| 22RV | 10:1 | 68.24 ± 1.24 [▲] | 68.16 ± 0.82 [▲] | 67.89 ± 0.94 [▲] | 58.81 ± 0.48 | 56.34 ± 1.23 | 29.85 ± 0.38 |
| | 20:1 | 77.59 ± 0.58 [▲] | 76.15 ± 0.71 [▲] | 77.64 ± 0.41 [▲] | 71.52 ± 0.74 | 71.87 ± 1.75 | 33.46 ± 1.38 |
| | 40:1 | 89.63 ± 0.93 ^{▲*} | 83.50 ± 0.85 [▲] | 83.14 ± 1.52 [▲] | 79.66 ± 0.64 | 74.50 ± 1.45 | 37.98 ± 0.49 |

[▲] $P < 0.05$ vs Ad-GFP-DC, DC-CTL, CTL group; * $P < 0.05$ CTL to LNCap vs DU145 or 22RV (at the same E:T); ^{▲*} $P < 0.05$ vs Ad-PSMA-DC-CTL or Ad-4-1BBL-DC-CTL group.

DISCUSSION

To date, DCs are the most powerful antigen-presenting cells that have been identified, and are a key link to the induction of specific immune response. However, because of the low immunogenicity of human malignancies, it has been difficult to identify an effective mechanism or specific antigen to activate the immune response against these malignancies, which has made vaccine therapies ineffective. In addition, it has not been possible to effectively present antigens (Smyth et al., 2001) as a result of low quantities of DCs and functional defects. The prostate-specific membrane antigen (PSMA) is a more sensitive and specific prostate cancer tumor marker compared to PSA and PAP, and exhibits highly specific expression in prostate cancer tissues making it one of the best options for targeted therapy (Wang and Mo, 2010). PSMA gene modified DCs enables the continuous expression of the PSMA antigen, generating

repeated CTL stimulation. It has been documented that certain cytokines can be secreted by prostate cancer cells to inhibit the activation of specific CTLs, which greatly impacts the efficacy of DC vaccines. A pressing problem, therefore, is how to enhance DC function to resist the effects of the prostate cancer microenvironment, which plays a key role in the efficacy of a DC vaccine (Pirtskhalaishvili et al., 2000; Kobie et al., 2003; Arlen and Gulley, 2005; Moon et al., 2008). 4-1BBL is a newly discovered costimulatory molecule expressed on the surface of antigen-presenting cells. 4-1BBL belongs to the TNF ligand superfamily, and can bind to 4-1BB molecules on the surface of T cells, generating costimulatory signals for late immune response, which is critical for survival and response of the CD8⁺ T cell mass (Sharma et al., 2010). This pair of co-stimulatory molecules play an important role in the immune response, immunomodulation, and anti-tumor and viral infection defense, in transplant rejection, etc., and have become an important area of modern immunological research. It has been discovered by Wiethe et al. (2003) that co-transfection of E7 and 4-1BBL genes into DCs mediated by recombinant adenovirus can greatly increase DC induced CTL specific killing effects of highly expressing E7 tumor cells (Wiethe et al., 2003). In this study we not only transfected PSMA into DCs, but also modify DCs with another cytokine gene, 4-1BBL, which led to not only increasing CTL targeted attacks on tumor cells or tissue, but also overcame poor expression of costimulatory molecules and the susceptibility to apoptosis. In addition to enhancement of the body's immune system, this therapy has the potential to bring new choices and a better potential for positive outcomes to prostate cancer treatment.

Adenovirus is the most highly effective and the most ideal (Zhang and Shen, 2003) vector currently in use for cancer associated antigen gene transfection. We transfected the *PSMA* and *4-1BBL* genes to immature DCs mediated by E1, E3 deficient adenovirus vectors, and demonstrated that both the endogenous antigen PSMA and 4-1BBL can be successfully expressed in DCs, and that they have no effect on DC differentiation and maturation. Furthermore, we demonstrated that co-expression resulted in an increase in the IL-12 secretory ability of DCs after transfection and better enhancement and generation of cytotoxic T lymphocytes (CTLs). We observed that the secretory content of IFN- γ in DC vaccine and CTL cell co-culture supernatant was higher than in the DC-CTL and CTL group, whereas the IL-10 group has the lowest content, suggesting that DC cell transfection induced the lymphocyte differentiation toward Th1, and sequentially greatly motivating antigen-specific CTL activity. In addition, we found that CTLs induced by virus transfected DCs had an obvious killing activity on PSMA highly expressing LNCap cells, but a lower killing activity on DU145 and 22RV prostate cancer cell lines, with the co-transfection group showing the highest activity among the different transfection groups. These *in vitro* experimental results allowed us to further uncover the molecular mechanism of DC functional status, providing an experimental and theoretical basis for a prostate cancer vaccine that could be useful for clinical gene therapy. This study also indicated that it is possible to get preferable therapeutic effects through immunization-mediated stimulation of nonspecific immune responses to maintain and enhance the body's specific anti-tumor immune responses.

REFERENCES

- Arlen PM and Gulley JL (2005). Therapeutic vaccines for prostate cancer: a review of clinical data. *Curr. Opin. Investig. Drugs* 6: 592-596.
- Dawicki W and Watts TH (2004). Expression and function of 4-1BB during CD4 versus CD8 T cell response *in vivo*. *Eur. J. Immunol.* 34: 743-751.

- Gu F (2000). Epidemiological survey of benign prostatic hyperplasia and prostate cancer in China. *Chin. Med. J.* 113:299-302.
- Jemal A, Siegel R, Ward E, Hao Y, et al. (2008). Cancer statistics, 2008. *CA Cancer J. Clin.* 58: 71-96.
- Kobie JJ, Wu RS, Kurt RA, Lou S, et al. (2003). Transforming growth factor beta inhibits the antigen presenting functions and antitumor activity of dendritic cell vaccines. *Cancer Res.* 63: 1860-1864.
- Moon C, Park JC, Chae YK, Yun JH, et al. (2008). Current status of experimental therapeutics for prostate cancer. *Cancer Lett.* 266: 116-134.
- Perner S, Hofer MD, Kim R, Shah RB, et al. (2007). Prostate specific membrane antigen expression as a predictor of prostate cancer progression. *Hum. Pathol.* 38: 696-701.
- Pirtskhalaishvili G, Shurin GV, Gambotto A, Esche C, et al. (2000). Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. *J. Immunol.* 165: 1956-1964.
- Sharma RK, Schabowsky RH, Srivastava AK, Elpek KG, et al. (2010). 4-1BB ligand as an effective multifunctional immunomodulator and antigen delivery vehicle for the development of therapeutic cancer vaccines. *Cancer Res.* 70: 3945-3954.
- Smyth MJ, Godfrey DI and Trapani JA (2001). A fresh look at tumor immunosurveillance and immunotherapy. *Nat. Immunol.* 2: 293-299.
- Wang Q (2003). Advances in 4-1 BBL. *Arch. Foreign Med. Immunol.* 26:304-307.
- Wang W and Mo ZN (2010) Advances in prostate-specific membrane antigen targeted therapies for prostate cancer. *Zhonghua Nan Ke Xue* 16: 547-51.
- Wiethe C, Dittmar K, Doan T, Lindenmaier W, et al. (2003). Provision of 4-1BB ligand enhances effector and memory CTL responses generated by immunization with dendritic cells expressing a human tumor-associated antigen. *J. Immunol.* 170: 2912-2922.
- Zhang Y and Shen X (2003). Common viral vector transfection effects on dendritic cells. *Arch. Foreign Med. Immunol.* 25: 188-190.