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Original Article

Efficacy of autologous tumor antigen sensitized dendritic cells in combination with cytokine induced killer cells on ovarian cancer immune therapy

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Abstract: Traditional cancer treatments, including surgery, chemotherapy, and radiotherapy, have the danger of metastasis, recurrence, normal tissue and immune function damage. Cellular immune therapy has become a hot spot in tumor treatment. This study selected ovarian cancer patients to extract autologous DCs and prepare Ag-DC-CIK, and further analyze its immunotherapy effect on ovarian cancer. Methods: Ovarian cancer cells were extracted from enrolled patients to prepare autologous antigen. DCs were sensitized to induce Ag-DC-CIK together with cytokine. Cell morphology was observed, cell phenotype was detected, and immune related indicators in peripheral blood were tested. Curative effect was evaluated and the adverse reaction was observed. DCs mature and keep suspension as dendritic following the incubation time. Ag-DC-CIK cells number increased obviously in spindle shape ($P < 0.05$). CD40, CD83, CD86, and HLA-DR levels were significantly higher in Ag-DCs than that in DCs on the seventh day ($P < 0.05$). Ag-DC-CIK presented higher CD3, CD3CD8, and CD3CD56 levels than CIK ($P < 0.05$). CD3, CD4, CD4/CD8, and CD56 in peripheral blood increased, while CD8 decreased in experimental group after two weeks treatment ($P < 0.05$). The efficiency (CR+PR+SD) was 95% in experimental group, which was higher than the control. The incidence of adverse reaction was lower than the control ($P < 0.05$). Autologous tumor antigen sensitized dendritic cells in combination with cytokine induced CIK combined passive and active immunotherapies, and showed better clinical efficacy and antitumor immunity than single CIK application.

Keywords: Autologous antigen, dendritic cell, killer cell, immunotherapy, ovarian cancer

Introduction

Ovarian cancer has high incidence and mortality that account for the second-highest in the female reproductive system tumor. A lot of patients are in advanced stage in treatment, and the recurrence rate is still high even after surgery and adjuvant chemotherapy [1]. Tumor immune mainly depends on the cellular immunity. The human body rejects malignant cells through immunologic mechanism. Malignant cells would form malignant tumor when escaping immune surveillance. Immunodeficiency may make the tumorigenesis difficult to avoid and hard to cure. Following surgery, chemotherapy, and radiotherapy, immune therapy is a new cancer treatment mode focusing on the low immunity. Biological treatment is aimed at the immune system that receiving more and more attention. Co-culturing monocytes in patients' peripheral blood and cytokines can obtain induced killer cells (CIK) [2]. Dendritic cells (DC) is a kind of antigen presenting cells (APC) that mainly induces immune response and antigen specific cytotoxic T lymphocyte (CTL) reaction [3]. DC and CIK are widely used on the tumor cell immune for their combination could guarantee the immune response kill tumor cells effectively. CIK immunotherapy is a hot spot in tumor biological immune in recent years mainly in the gastrointestinal tract malignant tumor, lung cancer, melanoma, prostate cancer, ovarian cancer, and other malignant tumors. CIK cells immunotherapy can kill the ovarian cancer cells directly or indirectly by regulating immune system. It can keep antitumor effects for long time by inducing tumor cells apoptosis. Compared with traditional antitumor method, such as radiotherapy, chemotherapy, and surgery, etc., it can obviously increase the five-year sur-

vival rate and disease-free survival. This study chose ovarian cancer patients to extract autologous DCs and prepare Ag-DC-CIK, and further analyze its immunotherapy effect on ovarian cancer.

Materials and methods

General information

40 cases of ovarian cancer patients in late stage between Jan 2013 and Jan 2015 with mean age at 52.4 ± 2.5 (35-76) years old from *CangZhou Central Hospital*. The study protocol was approved by the Research Ethics Committee of Cangzhou Central Hospital, and all patients gave their informed consent before study commencement. All patients were diagnosed in stage III/IV and the expected survival was longer than six months. All enrolled subjects showed normal organ function without serious infectious disease or allergy to biological products.

The patients were randomly equally divided into two groups. 20 patients in experimental group showed average age at 53.1 ± 2.3 (35-75) years old. Pathological staging: 6 cases in IIIA, 8 cases in IIIB, 3 cases in IIIC, and 3 cases in IV. Pathological type: 8 cases with serous cystadenocarcinoma, 5 cases with mucous cystadenocarcinoma, 4 cases with endometrial cystadenocarcinoma, 2 cases with clear cell carcinoma, and 1 case with mixed epithelial carcinoma. 20 patients in control group presented mean age at 54.2 ± 2.1 (35-73) years old. Pathological staging: 7 cases in IIIA, 5 cases in IIIB, 4 cases in IIIC, and 4 cases in IV. Pathological type: 9 cases with serous cystadenocarcinoma, 4 cases with mucous cystadenocarcinoma, 3 cases with clear cell carcinoma, and 4 cases with mixed epithelial carcinoma. There were significant differences in gender, age, stage, and pathological stage between two groups ($P > 0.05$).

Inclusion criteria: Patients received DC-CIK immunotherapy at least eight weeks after surgery, radiotherapy, or chemotherapy. Patients never received immunotherapy before. KPS score ≥ 60. Expected survival time > 3 months.

Exclusion criteria: Vital organs dysfunction including heart, liver, and kidney. Together with other malignant tumor. Systemic acute or chronic infectious disease. Spiritual or mental illness cannot cooperate with the treatment.

Instruments and reagents

Flow cytometry (BD, USA). Lymphocyte separation medium (Tianjin Chuanye biochemical products co., LTD.). Rat antihuman CD40, CD83, CD86, and HLA monoclonal antibody (eBioscience). Recombinant human granulocyte stimulating factor (CSF), interleukin-4 (IL-4), interferon-γ (IFN-γ), and interleukin-2 (IL-2) (Peprotech). RPM I1640 medium (Hyclone).

Methods

Autologous ovarian cancer cell preparation: Fresh ovarian cancer tissue was extracted from the patients and the cell suspension was prepared at 1×10^6/mL. Lymphocyte separation medium was added to the suspension and centrifuged at 3000 r/min for 20 min. Autologous ovarian cancer cells were obtained from the white layer on the interface.

Tumor antigen preparation: Autologous ovarian cancer cell was cultured in RPMI1640 medium to prepare tumor antigen. After frezen at -80°C, the cells were rewarmed to 37°C and disrupted by ultrasound. After centrifuged at 3000 r/min for 5 min, the cell lysis content was detected and stored at -80°C.

DC induction culture: PBMCs were collected from the patients and cultured in RPMI1640 medium under 37°C and 5% CO_2. The cells gradually grew by adherence, of which the suspension cells were the precursor cells of CIK. After adding CSF and IL-4, autologous ovarian cancer cell antigen was added on the fifth day to induce Ag-DC formation. They became mature on the seventh day.

DC phenotype determination: DC and Ag-DC cells on the fifth and seventh day were washed and centrifuged for CD40, CD83, CD86, and HLA-DR expression detection on flow cytometry.

Ag-DC-CIK cell induction culture: CIK precursor cells were washed and regulated at 1×10^6/mL. And then the cells were maintained at 37°C and 5% CO_2 in medium with IFN-γ (1000 μ/mL), CD3 monoclonal antibody (100 mg/mL), and IL-2 (1000 μ/mL). On the seventh day, CIK cells were divided into CIK cells untreated with Ag-DC and Ag-DC-CIK cells.

Ag-DC-CIK cell phenotype determination: CIK and Ag-DC-CIK cells were observed every day

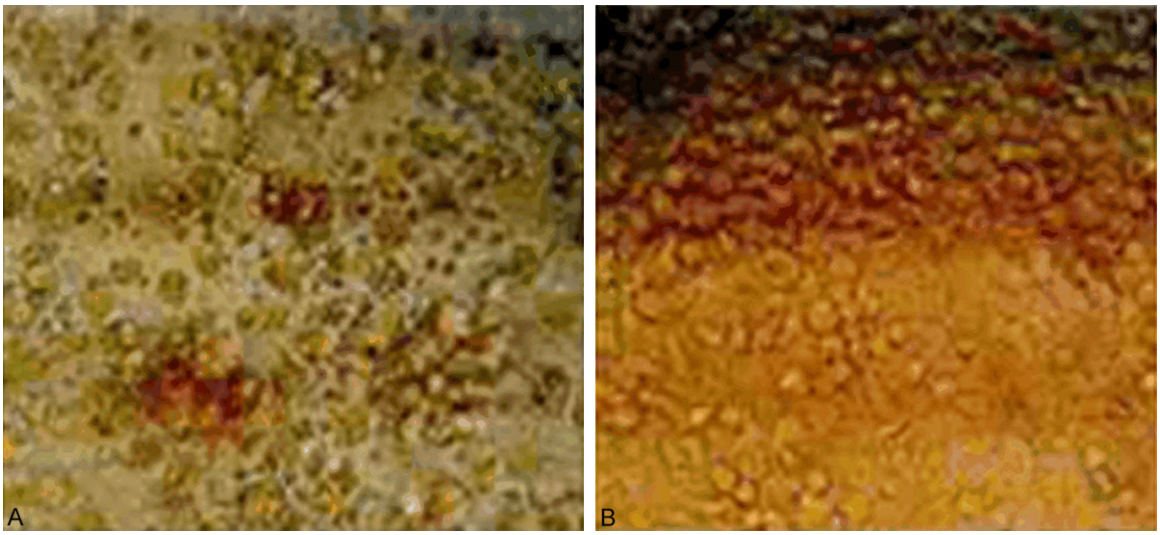

Figure 1. Cell culture and morphologic change (×400). A. DC after 14 days' cultivation; B. CIK after 14 days' cultivation.

Table 1. DC and Ag-DC cell phenotype detection

Item	DC	Ag-DC
CD40		
5th day	5.12 ± 1.47	47.32 ± 4.03
7th day	8.41 ± 2.01*	76.25 ± 4.67*,#
CD83		
5th day	1.15 ± 0.49	39.49 ± 10.28
7th day	2.68 ± 0.93*	63.73 ± 12.45*,#
CD86		
5th day	37.22 ± 5.33	61.43 ± 5.67
7th day	50.72 ± 8.57*	90.37 ± 6.43*,#
HLA-DR		
5th day	31.22 ± 7.12	70.28 ± 3.45
7th day	51.24 ± 10.36*	92.54 ± 4.79*,#

*$P < 0.05$, compared with the 5th day; #$P < 0.05$, compared with DC.

for CD3, CD4, CD8, CD4 CD8, and CD56 levels determination on flow cytometry.

Immunotherapy: Experimental group: Ag-DC-CIK cells were collected on the 14th day. Total cell number > 1×10^9, and the living cell proportion > 95% without pollution. The cells were maintained in 100 mL normal saline containing IL-2 and intravenous dripped for five continuous days. The patients received two cycles treatment including 28 days as a cycle.

Control group: CIK cells in equal number were suspended in 100 mL normal saline and intravenous dripped for five continuous days. The patients received two cycles treatment including 28 days as a cycle.

Immune status evaluation: 2 mL peripheral venous blood was extracted on the 7 days before treatment and 2 weeks after treatment. CD3, CD4, CD8, CD4, CD8, and CD56 expression were detected by flow cytometry.

Clinical curative effects evaluation: The patients were evaluated for curative effect after two cycles' treatment according to RECIST 1.1 solid tumor therapeutic effect evaluation standard [4]. Complete remission (CR): lesions disappeared without new ones, tumor markers normal for at least 4 weeks. Partial remission (PR): total lesions maximum diameter reduction ≥ 30% for at least 4 weeks. Stable disease (SD): total lesions maximum diameter reduction less than PR, or increase less than PD. Progression disease (PD): total lesions maximum diameter increase at least 20% or appeared new lesion.

Adverse reaction surveillance: Temperature, pulse, respiration and blood pressure changes of enrolled subjects were observed. Different types of adverse reaction were recorded, including leukopenia, thrombocytopenia, anemia, nausea, vomiting, and abnormal liver or renal function, etc. Blood routine and hepatorenal function were reviewed every week.

Data analysis

All the statistical analysis was performed on SPSS17.0 software. The data was presented

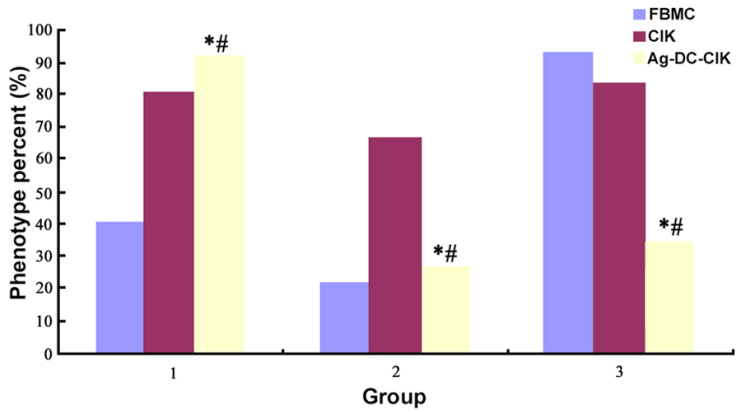

Figure 2. CIK and Ag-DC-CIK phenotype determination. 1: CD3; 2: CD3CD8; 3: CD3CD56. *P < 0.05, compared with PBMC; #P < 0.05, compared with CIK.

Table 2. Immune state comparison

Item	Experimental group	Control
CD3		
Before treatment	39.35 ± 8.65	18.24 ± 2.52
After treatment	65.49 ± 13.69*,#	46.13 ± 5.62*
CD4		
Before treatment	25.29 ± 3.78	10.41 ± 2.23
After treatment	32.98 ± 5.13*,#	16.71 ± 3.25*
CD8		
Before treatment	21.65 ± 4.78	17.42 ± 2.61
After treatment	16.24 ± 3.24*,#	9.73±3.21*
CD4/CD8		
Before treatment	1.32 ± 0.12	0.97 ± 0.05
After treatment	1.47 ± 0.35*,#	1.12 ± 0.07*
CD56		
Before treatment	22.14 ± 3.16	10.38 ± 2.46
After treatment	27.64 ± 3.73*,#	22.45 ± 3.07*

*P < 0.05, compared with before treatment; #P < 0.05, compared with control.

as Mean ± SD. t-test and chi-square test were used for mean comparison. P < 0.05 was considered as statistically significant.

Results

Cell culture and morphologic change

With the extension of incubation time, DCs became mature and grew in suspension. The cells presented dendritic type. Induced CIK cell number increased and volume enlarged in spindle shape. CIK cells proliferation became faster obviously since the 6th day and reached 9.7 ± 1.32 times on the 14th day. Ag-DC-CIK cells proliferation rate significantly quicker than CIK cells after 6 days and reached 17.6 ± 1.86 times on the 14th day (**Figure 1**).

DC and Ag-DC cell phenotype detection

DC and Ag-DC cells were collected on the 5th and 7th day for flow cytometry detection. It was found that CD40, CD83, CD86, and HLA-DR level in DC and Ag-DC on the 7th day were markedly higher than that on the 5th day (P < 0.05). On the 7th day, Ag-DC showed obviously higher level of CD40, CD83, CD86, and HLA-DR than DC (P < 0.05) (**Table 1**).

CIK and Ag-DC-CIK phenotype determination

The results revealed that after cytokine induction, CD3, CD3CD8, and CD3CD56 elevated significantly in CIK cells compared with PBMC (P < 0.05). While their levels were obviously higher in Ag-DC-CIK cells induced by cytokine than that in CIK (P < 0.05) (**Figure 2**).

Immune state comparison

The subjects in experimental group received Ag-DC-CIK cell infusion treatment, while the control group received CIK cell infusion. Peripheral blood was extracted for immune index detection at 7 days prior to treatment and 7 days after the second treatment cycle. It was found that CD3, CD4, CD4/CD8, and CD56 increased, while CD8 decreased after treatment (P < 0.05). Compared with control, experimental group showed elevated CD3, CD4, CD8, CD4/CD8, and CD56 level in peripheral blood (P < 0.05) (**Table 2**).

Curative effect comparison

Curative effect evaluation showed that CR accounted for 30%, PR accounted for 45%, SD accounted for 20%, and PD accounted for 5% in experimental group. The effective rate (CR+PR+SD) was 95% in experimental group,

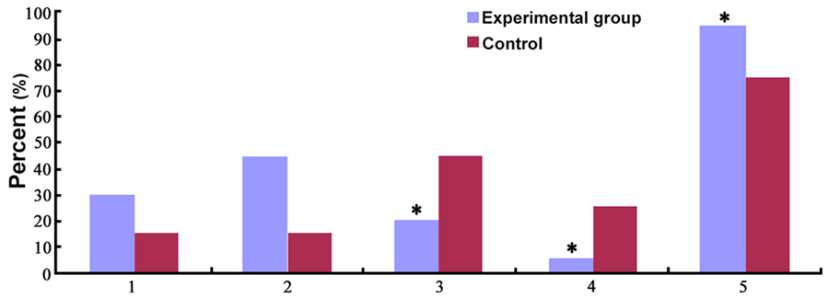

Figure 3. Curative effect comparison. 1, CR; 2, PR; 3, SD; 4, PD; 5, effective rate *P < 0.05, compared with control.

Table 3. Adverse reaction comparison

Adverse reaction	Experimental group (cases)						Control (cases)					
	0	I	II	III	IV	Incidence	0	I	II	III	IV	Incidence
leukopenia	10	4	5	1	0	50%*	3	6	6	3	2	85%
Thrombocytopenia	8	8	3	1	0	50%*	4	7	6	2	1	80%
Anemia	10	5	4	1	0	60%*	2	5	8	3	2	90%
Nausea and vomiting	10	8	2	0	0	50%*	3	6	7	2	2	85%
Abnormal liver function	10	8	2	0	0	50%*	3	5	7	2	3	85%
Abnormal kidney function	10	7	2	1	0	50%*	4	5	6	2	3	80%

*P < 0.05, compared with control.

which was markedly higher than that of control as 75% (**Figure 3**).

Adverse reaction comparison

Adverse reaction observation indicated that some patients appeared leukopenia, thrombocytopenia, anemia, nausea, vomiting and abnormal liver and kidney function. Adverse reaction incidence in experimental group was markedly lower than control (P < 0.05) (**Table 3**).

Discussion

Ovarian cancer is a common tumor in our country with high incidence and mortality rate that seriously threatens women's health and quality of life [5]. Surgery together with platinum chemotherapy and radiotherapy is the first choice for ovarian cancer treatment. However, its effect is not ideal because of the easy recurrence and metastasis of ovarian cancer [6]. In recent years, immunotherapy as the fourth kind of treatment became the hot spot in ovarian cancer. It can improve the tumor antigen recognition ability and kill ability, break the immune tolerance and immune suppression, and delay tumor recurrence to increase the curative rate and patient's survival rate [7].

DC in combination with cytokine induced CIK immunotherapy is a new treatment that can kill tumor cells and reduce the tumor recurrence rate without obvious adverse reaction [8]. After first found in 1973, DC can absorb and identify tumor antigen, further present to T cells and induce T lymphocytes response. DC load autologous tumor antigen can induce CTL with specific anti-tumor ability [9, 10]. CIK is a type of immune effector cell with strong proliferation ability and cell toxicity. It has cytolysis effect and can remove the residual tumor foci in patients to prevent relapse. It also has the non-MHC restriction antitumor activity [11, 12].

In this study, ovarian cancer patients in our hospital were selected for ovarian cancer cell isolation. Tumor antigen was prepared to induce DC. Morphological observation showed that following the extension of incubation time, DCs became mature and grew in suspension. The cells presented as arborization. Induced Ag-DC-CIK cell number increased and volume enlarged in spindle shape. It was found that CD40, CD83, CD86, and HLA-DR level in DC and Ag-DC on the 7th day were markedly higher than that on the 5th day. On the 7th day, Ag-DC showed obviously higher level of CD40, CD83, CD86, and HLA-DR than DC, while Ag-DC-CIK presented higher expression of CD3, CD3CD8, and CD3CD56 than CIK. Matured Ag-DC expressed higher level of costimulatory molecules like CD40, CD83, CD86, and HLA-DR to improve the efficiency of antigen presentation, promote Ag-DC-CIK cells proliferation activity, and enhance the antitumor activity [13].

Sensitizing autologous DCs by tumor antigen and co-culturing with CIK cells can produce dual antitumor effect with specific features [14]. CD3 and CD8 are key factors involved in the innate immune response, while CD56 is a characteristic symbol of natural killer cells that mainly reflects the nonspecific immune response [15]. This study divided subjects into two groups randomly. The subjects in experimental group received Ag-DC-CIK cell infusion, whereas the control group received CIK cell infusion. Immune index detection at 7 days prior to treatment and 7 days after the second treatment cycle showed that CD3, CD4, CD4/CD8, and CD56 increased, while CD8 decreased after treatment. It suggested that autologous tumor antigen sensitized dendritic cells in combination with cytokine induced CIK immunotherapy can activate CIK cells and increase the activity of natural killer cells. Ag-DC-CIK cell infusion can increase the ovarian cancer patients' immune function including both specific and non-specific immune response. Studies suggested that tumor patient presented immune dysfunction and immune classification proportion changes in peripheral blood T lymphocytes, including CD4, CD4/CD8 reduction and Th1 shift to Th2 cytokines [16]. Autologous tumor antigen sensitized DC in combination with CIK cells can correct immune dysfunction by overexpressing MHC-I and MHC-II antigen presenting molecules. It can overexpress stimulating factors such as CD83 and CD86, activate T cells, and enhance T lymphocyte recognition and killing function [17]. In addition, mature DC can secrete a variety of cytokines to promote NK cell activation [18].

This study evaluated the curative effect after 2 cycles' treatment. The effective rate (CR+PR+SD) in the experimental group was 95%, which was higher than that of control (75%). On the other side, patients appeared multiple adverse reaction including leukopenia, thrombocytopenia, anemia, nausea, vomiting, liver dysfunction, and renal dysfunction. Experimental group presented significant lower adverse reaction rate than the control, indicating that autologous tumor antigen sensitized DC combined cytokines induced CIK immunotherapy can obviously improve the treatment effect and reduce the occurrence of adverse reactions. Li thought that DC-CIK cells in combination with chemotherapy can increase about 16% on non-small cell lung cancer 2-year survival rate [19]. Through collecting autoantigen, sensitizing immune cells, and combining cytokines to induce killer cells, biological treatment can markedly improve the clinical therapeutic effect, alleviate tumor associated symptoms, and prolong patients' survival time with minor adverse reaction. It showed unique anti-tumor advantage and good security [20]. Our results were similar with previous reports.

Above all, autologous tumor antigen sensitized dendritic cells in combination with cytokine induced killer cells combined passive and active immunotherapy, and presented better clinical curative effect and antitumor immune responses compared with single CIK application. Ag-DC-CIK cells showed safe, effective, and feasible characteristics on advanced ovarian cancer patients individualized treatment. Further investigation about autologous tumor antigen purification, concentration, and proportion is needed to provide theoretical basis for clinical extensive application.

Disclosure of conflict of interest

None.

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References

[1] Brenner MK and Heslop HE. Adoptive T cell therapy of cancer. Curr Opin Immunol 2010; 22: 251-257.

[2] Mesiano G, Todorovic M, Gammaitoni L, Leuci V, Giraudo Diego L, Carnevale-Schianca F, Fagioli F, Piacibello W, Aglietta M and Sangiolo D. Cytokine-induced killer (CIK) cells as feasible and effective adoptive immunotherapy for the treatment of solid tumors. Expert Opin Biol Ther 2012; 12: 673-684.

[3] Cruz CR, Hanley PJ, Liu H, Torrano V, Lin YF, Arce JA, Gottschalk S, Savoldo B, Dotti G, Louis CU, Leen AM, Gee AP, Rooney CM, Brenner MK, Bollard CM and Heslop HE. Adverse events following infusion of T cells for adoptive immunotherapy: a 10-year experience. Cytotherapy 2010; 12: 743-749.

[4] Rozkova D, Tiserova H, Fucikova J, Last'ovicka J, Podrazil M, Ulcova H, Budinsky V, Prausova J, Linke Z, Minarik I, Sediva A, Spisek R and

Bartunkova J. FOCUS on FOCIS: combined chemo-immunotherapy for the treatment of hormone-refractory metastatic prostate cancer. Clin Immunol 2009; 131: 1-10.
[5] Bernatchez C, Radvanyi LG and Hwu P. Advances in the treatment of metastatic melanoma: adoptive T-cell therapy. Semin Oncol 2012; 39: 215-226.
[6] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.
[7] Montagna D, Turin I, Schiavo R, Montini E, Zaffaroni N, Villa R, Secondino S, Schiavetto I, Caliogna L, Locatelli F, Libri V, Pession A, Tonelli R, Maccario R, Siena S and Pedrazzoli P. Feasibility and safety of adoptive immunotherapy with ex vivo-generated autologous, cytotoxic T lymphocytes in patients with solid tumor. Cytotherapy 2012; 14: 80-90.
[8] Wathelet N and Moser M. Role of dendritic cells in the regulation of antitumor immunity. Oncoimmunology 2013; 2: e23973.
[9] Hargadon KM. Tumor-altered dendritic cell function: implications for anti-tumor immunity. Front Immunol 2013; 4: 192.
[10] Kandalaft LE, Chiang CL, Tanyi J, Motz G, Balint K, Mick R and Coukos G. A Phase I vaccine trial using dendritic cells pulsed with autologous oxidized lysate for recurrent ovarian cancer. J Transl Med 2013; 11: 149.
[11] Rajbhandary S, Zhao MF, Zhao N, Lu WY, Zhu HB, Xiao X, Deng Q and Li YM. Multiple cytotoxic factors involved in IL-21 enhanced antitumor function of CIK cells signaled through STAT-3 and STAT5b pathways. Asian Pac J Cancer Prev 2013; 14: 5825-5831.
[12] Yang B, Wang J, Cai LL, Zhu HL, Yu RL, Chi XH and Lu XC. Treatment of multiple solitary plasmacytomas with cytokine-induced killer cells. Cytotherapy 2014; 16: 278-284.
[13] Cui Y, Yang X, Zhu W, Li J, Wu X and Pang Y. Immune response, clinical outcome and safety of dendritic cell vaccine in combination with cytokine-induced killer cell therapy in cancer patients. Oncol Lett 2013; 6: 537-541.
[14] Wongkajornsilp A, Somchitprasert T, Butraporn R, Wamanuttajinda V, Kasetsinsombat K, Huabprasert S, Maneechotesuwan K and Hongeng S. Human cytokine-induced killer cells specifically infiltrated and retarded the growth of the inoculated human cholangiocarcinoma cells in SCID mice. Cancer Invest 2009; 27: 140-148.
[15] Zhan HL, Gao X, Pu XY, Li W, Li ZJ, Zhou XF and Qiu JG. A randomized controlled trial of postoperative tumor lysate-pulsed dendritic cells and cytokine-induced killer cells immunotherapy in patients with localized and locally advanced renal cell carcinoma. Chin Med J (Engl) 2012; 125: 3771-3777.
[16] Shah W, Yan X, Jing L, Zhou Y, Chen H and Wang Y. A reversed CD4/CD8 ratio of tumor-infiltrating lymphocytes and a high percentage of CD4(+)FOXP3(+) regulatory T cells are significantly associated with clinical outcome in squamous cell carcinoma of the cervix. Cell Mol Immunol 2011; 8: 59-66.
[17] Chang JM, Hung LM, Chyan YJ, Cheng CM and Wu RY. Carthamus tinctorius Enhances the Antitumor Activity of Dendritic Cell Vaccines via Polarization toward Th1 Cytokines and Increase of Cytotoxic T Lymphocytes. Evid Based Complement Alternat Med 2011; 2011: 274858.
[18] Chau J, Moza D, Hossain N, Lee JK, Bienenstock J and Karimi K. Increased production of IFN-gamma by natural killer cells triggered with bone marrow-derived dendritic cells cultured in the presence of retinoic acid. Eur J Pharmacol 2013; 715: 321-327.
[19] Li H, Wang C, Yu J, Cao S, Wei F, Zhang W, Han Y and Ren XB. Dendritic cell-activated cytokine-induced killer cells enhance the anti-tumor effect of chemotherapy on non-small cell lung cancer in patients after surgery. Cytotherapy 2009; 11: 1076-1083.
[20] Kimura H, Iizasa T, Ishikawa A, Shingyouji M, Yoshino M, Kimura M, Inada Y and Matsubayashi K. Prospective phase II study of post-surgical adjuvant chemo-immunotherapy using autologous dendritic cells and activated killer cells from tissue culture of tumor-draining lymph nodes in primary lung cancer patients. Anticancer Res 2008; 28: 1229-1238.