Clinical evaluation of autologous DC and CIK cell therapy combine with chemotherapy in lung cancer patients

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Abstract: Clinical efficacy of immunotherapy using dendritic cells (DC) and cytokine induced killer cells (CIK), combined with chemotherapy was observed in lung cancer patients. Thirty five patients with lung cancer, who admitted to the Second Affiliated Hospital of Zhengzhou University from August 2012 to June 2013 were treated with the regimen of DC-CIK based immunotherapy combined with chemotherapy. Healthy control (n = 6) were age-and sex-matched. The ratio of CD4+CD25+ FoxP3+ (Treg), CD8+CD28−(Ts) and CD8+CD28+(CTL) were analyzed using flow cytometry. Patients received 4~8×10^7 DCs and 5~10×10^9 CIKs in total during the treatment courses. There was an increasing tendency for proportions of T subsets and NK cells, and decreased tendency for tumour markers in peripheral blood after treatment though no statistical significance. CR was found in 3 cases (8.6%), PR in 8 cases (22.9%), SD in 21 cases (60%) and PD in 3 cases (8.5%) within 1 year follow-up. Patients showed significantly higher percentage of Treg and lower level CTL in peripheral blood compared to healthy control ($P<0.05$), and recovered to similar level as healthy people. No serious adverse reaction was found in all patients. We can conclude that DC-CIK cellular immunotherapy combined with chemotherapy is beneficial for lung cancer in improving quality of life, and reducing levels of Treg in peripheral blood without serious side effect.
[Haixia Dong, Yanjie Sun, Zhongmian Zhang, Haoxun Wang, Na Han, Weiwei Tian, Ping Liu, Jian wang, Haili Wang, Xianrui Song, Haiyan Kong, Changwei Feng, Yin Li, Jun Ma. **Clinical evaluation of autologous DC and CIK cell therapy combine with chemotherapy in lung cancer patients.** *Life Sci J* 2014;11(7):455-458]. (ISSN:1097-8135). http://www.lifesciencesite.com. 55

Keywords: Adoptive cellular immunotherapy; Dendritic cells; Cytokine-induced killer cells; lung cancer; Chemotherapy

1.Introduction

Lung cancer accounts for about 13% of total deaths, which represents the highest incidence and mortality among malignant tumours. Despite recent advances in therapies of surgery, chemotherapy, radiotherapy and other treatments for lung cancer, clinical outcomes remain poor. The five-year survival rate for lung cancer is still less than 15% (Jemal et al., 2008). Traditional treatment cannot completely kill tumour cells. Instead, it has serious side effects to patients and reduce the immune function of patients against tumour, which is one of the causes for tumour recurrence and metastasis.

Immunotherapy in recent years has developed a very promising treatment method, including active immune cell therapy such as dendritic cells (DC) vaccine, and adoptive immune cell therapy, such as cytokine-induced killer (CIK) cells. DCs can induce cytotoxic T lymphocyte immune responses against cancer cells. Encouraging clinical results of DCs based regimens in treatment of lung cancer have been reported. CIK therapy, with advantages of fast proliferation of immune cells, strong anti-tumour activities, broad spectrum anti-tumour activities, secreting a variety of cytokines and sensitive to multi-drug resistant tumour cells, is considered to play an important role in treatment of lung cancer (Schmidt-Wolf et al., 1991).

The purpose of this study was designed to evaluate the immune responses and its clinic outcomes with DC-CIK therapy plus chemotherapy in lung cancer patients.

2. Patients and methods

Patients

Retrospective study was performed at Department of Cancer Biotherapy, the Second Affiliated Hospital, Zhengzhou University. Thirty five patients with lung cancer were admitted to the department and received

DC-CIK therapy combined with chemotherapy from August 2012 to June 2013. All patients were received informed consent under a protocol reviewed and approved by the our hospital. The regimen of chemotherapy were Gemcitabine combined with carboplatin (29 cases) or Irinotecan combined with carboplatin (6 cases). Among the 35 cases, 3 cases received two courses and the other 32 cases received one course of DC-CIK therapy. All patients were selected without serious liver and renal dysfunction, and Karnofsky performance status score (KPS) were more than 50 points in all cases.

Preparation of DC and CIK

Blood cells in 50~80 ml plasma were collected underwent leukapheresis using the Fresenius KABI System, and PBMCs were isolated from the interface layer by Ficoll-Hypaque gradient centrifugation. About 1×108 cells were collected for each patient.

PBMCs were washed twice with normal saline by centrifugation. The supernatants were removed and remains were re-suspended with serum-free AIM-V medium (Gibco). The cells at a concentration of 2~4×106/ml were plated into 6-well plates, cultured at 37℃ with 5% CO2 for 2 hours. Then the cells were washed 2 times with serum-free AIM-V medium, cultured with DC medium for 3 days, and continuously cultured with the supplemented DC medium for another 2 days. The cells were pulsed in the presence of whole lung cancer specific antigens which was obtained from the crushed supernatant with corresponding tumor cell lines or resected tumor tissue for 12-16 hours, and cultured DCs were harvested on day 7.

For CIKs, collected mononuclear cells by centrifugation, counted and plated into culture flasks with the CIK initial medium at the concentration of 2×106/ml and placed at 37℃ with 5% CO2. CIK medium was supplied on the next day; after then, sampled and counted every 2 to 3 days, maintained the cells at 1~4×106/ml by supplementing culture medium or subculture. The cells were harvested between the 10th day to 17th day.

Quality control of DC and CIK

The cell viabilities of DC or CIK were above 90% with total amounts of 4×107 or 5×109, respectively. Immunophenotype of the cells was analyzed by flow cytometry. Cells were confirmed to be not contaminated by test of bacteria, fungi, mycoplasma and endotoxin before infusion. Which CD3+CD56+cells were above 20%. The quality of all test results are in accord with the " guiding principle of human somatic cell therapy and gene therapy formulated by the USA FDA.

Cell administration, and observation of clinical efficacy and side effect

Patients completed infusion in 1~2 hours each day for 4~5 days in each course, with 4 doses of DC (1x107) and 5 doses of CIK (1x109) infusion by intravenous. Patient received 1~2 courses in our observation period. We observed patients for at least 30 minutes after each infusion.

In the period of treatment, symptoms, physical signs, and side effects were recorded in detail. Parameters of lymphocyte subsets and function of liver and kidney were observed. Adverse reactions were evaluated according to the National Cancer Institute's patient-reported outcomes version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE) (Chen et al., 2012).The clinical effects were evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) (Zhong et al., 2011).

Flowcytometry analysis

Peripheral blood samples drawn into heparinized tubes were studied while still fresh. The first was surface staining. FoxP3 was intracellular staining. FoxP3 staining was performed using the FoxP3 staining buffer and the intracellular staining protocol. In brief, 1)after surface staining for 30 minutes at 4°C, cells were washed and incubated with freshly prepared fixation/permeabilization solution for 40 minutes at 4°C. 2) wash twice with permeabilization buffer. 3) stain with Ab to FoxP3 for 20 minutes at 4°C.Sample data were acquired FACSCanto cytometer (BD Biosciences) and analyzed using CellQuest analysis programme (Becton Dickinson). Markers of mature DC cells were detected by using CD83, CD86, CD40 and HLA-DR. Markers of CIK were CD3 and CD56. T cell subsets were detected using fluorescence labeled antibodies (Multitest IMK kit for T subsets, CD3/CD4/CD25/FoxP3 for Treg, CD3/CD8/CD28 for Ts. All reagents were purchased from BD Biosciences) (USA).

Statistical analysis

SPSS 19.0 was employed for statistical analysis. The differences of lymphocyte subsets and tumor markers between pre-treatment and post-treatment were analyzed by student's t tests.

3. Results

Patient characteristics

A total of 35 lung cancer patients including small cell lung carcinoma (17.1%), adenocarcinoma (68.6%), squamous lung carcinoma (14.3%) were included in the present study. Patients' age range 40~89 years (60.23±11.11). There were 26 male and 9 female patients. Among the 35 cases, 31 (88.6%) had tumor burden in their body and 31 (88.6%) received concurrent chemotherapy.

Efficacy of DC-CIK combined with chemotherapy

Percentages of mature DC markers were more than 70% for all those listed above, and CIK

(CD3+CD56+) were (23.91±6.69)%. There was an increase tendency for CD3+, CD3+CD8+, CD3+CD4+ and NK (CD16+CD56+) subsets in peripheral blood after treatment compared to those before treatment, but no significance observed. Decrease tendency of CA199, CA153, CEA, CA125 and TAM after treatments were also found in peripheral blood (Table 1).

Table 1: Changes of tumor markers after treatment

Group	Before treatment	After treatment	P
CA199	14.48±13.82	14.39±11.52	0.989
CA125	29.61±11.29	24.59±22.75	0.719
CA153	14.49±22.84	8.14±9.87	0.435
CEA	8.32±9.63	6.19±7.53	0.588
TAM	100.99±17.33	98.44±22.52	0.712

Among the 35 cases (Table 2), the total symptoms improvement rate was 91.4% (32/35), including cough and haemoptysis reduced in 17 cases (48.6%), chest distress eased in 9 cases (25.7%), haemoptysis disappeared in 2 cases (5.7%) and pain relief in 4 cases (11.4%). According to RECIST, there were 3 complete response cases (8.6%), 8 partial response cases (22.9%), 21 stable disease cases (60%) and 3 cases with progressed disease (8.5%). Fever (4 cases), bone marrow depression (3 cases), renal function (2 cases) and liver function abnormal (2 cases) were found, but all were mild.

We found significantly higher percentage of Treg and lower level of CTL in preipheral blood in patients compared to healthy control ($P<0.05$). After treatment, Treg decreased and CTL increased obviously (Table 3).

Table 2: Clinical outcome of lung cancer patients

	n	CR	PR	SD	PD
Pathological types					
SCLC	6	0	3	2	1
NSCLC	29	3	5	19	2
Clinical stages					
I-IIIA	20	2	6	12	0
IIIB-IV	15	1	2	9	3

Table 3: Changes of ratio of T cell subsets in peripheral blood in patients (%)

	lung cancer		Healthy control	P*
	before	After		
Treg	62.8±22.6	54.7±19.5	40.0±8.6	0.038
Ts	26.9±9.4	25.3±10.1	18.1±8.2	
CTL	20.1±4.1	29.5±9.8	28.7±6.0	0.016

* For healthy control，compared to those before treatment in lung cancer patients

4. Discussion

With the high morbidity and mortality, lung cancer whose mortality increases the most rapidly, has become one of the most dangerous malignancies. In worldwide, more than one million people died of lung cancer annually. The therapies of lung cancer have improved in recent years, but 5-year survival rate of patients is still less than 15%. Therefore it is of great significance to find new treatments(Pao and Girard,2011).

The incidence, development and prognosis of tumor are closely related to immune function. Low immune function and reduced immune surveillance function promote the occurrence and development of lung cancer(Elgert et al., 1998). As a result, the immunotherapy gradually becomes an important part of comprehensive treatment of lung cancer. DC is one of the most powerful antigen-presenting cell, with abundant molecules for antigen presenting on the surface (e.g. MHC I, MHC II, costimulatory molecules B7-1, B7-2, CAM-1, CAM-3, LFA-1 and LFA-3). It can effectively activate the initial T cells and trigger the body's anti-tumor immune response (Thanendrarajan et al., 2011). CIK cells improve immune response to suppress or eliminate tumor. On one hand, they directly kill target cells by releasing cytotoxic cytoplasma granula; on the other hand, they secrete various types of anti-tumor cytokines, such as IL-2, IFN-γ, TNF-α and so on. These cytokines not only inhibit tumor cell activity directly, but also indirectly kill tumor cells by regulating the body's immune reactivity. CIK cells can also express FasL which combines with Fas expressed in tumor cell membrane and consequently induces apoptosis of tumor cells. Gritzapis et al. (2002),Thorne et al.(2006) and Marin et al. (2006) have proven that intravenous-injected CIK cells can migrate to the tumor site and kill tumor cells at local in animal experiments.

The immunological competence of lung cancer patients and the mechanisms leading to disease progression are currently widely analyzed. Results from animal studies done by Terabe et al. (2004) and Matsumoto et al.(2011), support the possibility that one mechanism of cancer immune evasion may be related to the suppression of anti-tumor immune responses by regulatory T cells (Tregs). Studies presented that Treg cells suppressed the antitumor immune response, and their presence at local tumor sites correlates with an unfavorable prognosis. It has been shown that CD8+CD28− cells(Ts) also have a suppressive effect by Yao et al.(2012) and Filaci et al.(2007). These cells suppressed the cytotoxic function of T lymphocytes, and inhibited the T lymphocyte proliferation. In our study, Ts and Treg of lung patients group decreased after treatments. Autologous DC and CIK cell therapy combine with chemotherapy may reduce the level of Treg and Ts of lung cancers. O.V. Skachkova (2013) found that the number of NK-cells, CD4/CD8 ratio, MIP-1α and RANTES mRNA expression levels are important immunological markers associated with

favorable clinical outcome for NSCLC patients treated with DC-vaccine. It was necessary that found informative immunological parameters associated with clinical outcome during DC-CIK therapy. Treg and Ts may be immunological parameters to evaluate the clinical effect.

Cao S. et.al (2013) had observed the curative of CIK cell in NSCLC patients and the follow-up time was as long as seven years. The results show that CIK treatment can significantly prolong overall survival of NSCLC patients (24 months vs 10 months, $P < 0.001$) compared with the group which is only treated with chemotherapy. CIK treatment can delay the progress of advanced stage cancer. Our results showed that 32 (91.4%) patients were relieved of cough, hemoptysis and chest stuffiness, indicating that DC-CIK therapy combined with chemotherapy can obviously ameliorate patients' clinical conditions. The CD3, CD3CD4, CD3CD8 and CD16CD56 positive subsets in peripheral blood were raised after therapy suggested improved immune system function might involved in its therapeutic effect. The classified statistic analysis shows that DC-CIK therapy may effectively suppress the disease progression (disease control rate > 70%). Our study also demonstrated that immunotherapy has obvious efficacy on improving the quality of life and decreasing tumor marker levels and reducing levels of Treg in peripheral blood without serious side effect.

Acknowledgement:

Cell culture technology in this research was supported by Shanghai Clasion Biotechnology Co.

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4/15/2014