RESEARCH ARTICLE

Moderating Effects and Maintenance of Lung Cancer Cellular Immune Functions by CIK Cell Therapy

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Abstract

Aims: To study the CIK cell treatment effects on regulation of cellular immune function disorders in patients with lung cancer, and to analyze the time characteristics. Methods: Cellular immune function was assessed by FCM, and patients with functional disorders were randomly divided into two groups, one given CIK cell therapy within 18 months (5 courses) and the other the controls, which were followed up for 1 year with cellular immune functions tested once a month. Results: There were 5 types of cellular immunity, 4 of which are disorders; after CIK treatment, the improvement rate of the 4 groups were 79.1%, 70.8%, 76.0% and 70.0%, intergroup differences not being statistically significant (*P*=0.675), all significantly higher than in the control group (*P*=0.000). The median maintenance times for the 4 groups were 10.4 months (9.76-11.04), 8.4 months (7.86-8.94), 9.8 months (9.20-10.4) and 7.9 months (6.25-9.55), respectively. Conclusions: CIK cells were able to improve the immune functions of patients with lung cancer, the rate of improvement and maintenance time being related to the immune function before the treatment and CIK-cell-therapy courses.

Keywords: CIK cells - lung cancer - cellular immune disorders - improvement rate - maintenance time

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Introduction

With the in-depth studies of the molecular mechanisms of happening and development tumor and the development of biotechnologies, the biological treatment has become a new therapeutic approach following the surgical treatment, chemotherapy and radiation therapy treatment. Lung cancer is the most common malignant tumor in current world, its occurrence and development are closely related to the body's immune function status, and cell-mediated immunity is the main way for the anti-tumor immune (Wu et al., 2004). The tumor immunotherapy is the primary means of Cancer Biotherapy, and adoptive immunotherapy is an effective method of it (Urbanska et al., 2012). Cytokine-induced killer (CIK) has both the advantages of T lymphocytes' powerful anti-tumor activity and NK cells' killing tumor in non-MHC-restriction. It could directly kill tumor cells, adjust and enhance immune function, withnot damaging the structure and functions of the immune system. Its effects on the treatment of malignant solid tumors has been widely recognized (Blattman and Greenberg, 2004; Kim et al., 2010). But there is rarely reports on the analysis of the effects of regulation and maintaining time of cellular immune function in patients with lung cancer by CIK cell therapy, thus the author summarized and analyzed the experience of extracting the mononuclear cells (PMBC) to prepare CIK cells from the peripheral blood of patients with lung cancer, and the clinical efficacy data of prepared CIK cells reinfusion, reported as the following.

Materials and Methods

Studying objects

There was a total of 511 cases lung cancer patients in our hospital by out-patient and in-patient treatment from Jan. 2002 to Jan. 2012, age 24 to 69 years, average age 59.2±15.1 years. All patients were pathologically confirmed, and the normal control group, 200 cases, were healthy subjects randomly selected, aged 25 to 68 years, average age 45.2±11.3 years, and these subjects were excluded: (1) consolidated immune system disease; (2) combined with chronic wasting disease and infectious diseases; (3) combined with other malignancies.

Main instruments and reagents

CS-3000PLUS type blood cell separator machine was purchased from Baxter, Forma311 carbon dioxide incubator, Forma205 biological safety cabinets, Gibco serum-free medium (AMV), BECKMAN COULTER Epics XL flow cytometer, and the flow detection reagents CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5, CD3-FITC/CD16 +56- PE + CD45-PC5 were purchased from IMMUNOTECH Company.

Methods

Conventional-treatment group: All patients were given standardized chemotherapy after surgery.

CIK cell therapy group: All patients were given standardized chemotherapy for 1 month right after surgery, then autologous immune cells in vitro expansion and

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Table 1. Characteristics of Immune Function of Patients with Lung Cancer (x±SD)

Groups	N (%)	CD3 (%)	CD4 (%)	CD8 (%)	CD4/CD8	NK (%)	CIK (%)
Normal	81	69.6±7.28	37.8±5.45	23.7±3.96	1.63±0.33	12.4±5.24	3.17±1.87
Cancer	511	62.3±13.1	30.3±9.89	23.9±9.94	1.53±0.99	16.4±9.49	3.29±2.15
A	147 (28.8)	68.4±7.25	36.6±5.06	23.4±4.43	1.62±0.40	12.2±5.59	3.32±2.14
B	128 (25.0)	49.0±9.00	25.2±6.56	16.7±4.59	1.58±0.54	22.7±11.3	3.49±2.37
C	142 (27.8)	62.8±12.1	21.7±6.66	31.1±7.80	0.72±0.20	17.5±9.40	3.34±2.11
D	68 (13.3)	64.4±9.76	42.6±7.45	14.7±4.48	3.20±1.42	13.7±6.85	3.13±2.08
E	26 (5.1)	85.0±5.57	34.4±4.50	47.1±6.90	0.75±0.16	11.3±4.49	2.18±1.16

reinfusion were carried out.

CIK cell preparation: Mononuclear cells (PBMCs) were collected from the peripheral blood of patients with a blood cell separator, and suspended in serum-free medium, adjusting the cell density to 1×10^6/ml. Cells were seeded in culture flasks and incubated in 37 °C and 5% CO2 incubator train, 1 000 U/ml γ-IFN cultivation was added at 0 h, and 300 U/ml IL-2 and 50 ng/ml CD3McAb were added at 24h. Culture medium was replaced every 4 d, with adjusting the cell density to 1×10^6/ml and adding additional 300 U/ml IL-2, and 50 ng/ml CD3McAb was supplemented at the same time when IL-2 was complemented every 8 d. Cells were collected about 1 week.

CIK cell Reinfusion: At the end of cell culture, collected the cells with negative results of microorganisms testing, after centrifuged to remove culture medium, the cells were washed 3 times with physiological saline, then suspended and vein-reinfusioned in liquid dubbed with 30 ml 20% human serum albumin and 100 ml normal saline, once a day for 3consecutive days (as a course of treatment), the number of cells was in 2×10^9 to 6×10^9/ml in each reinfusion.

The detection method of the cellular immune: 100ul fresh EDTA-anticoagulated peripheral blood was taken, added 20ul monoclonal antibody, mixed and stained in darkness at room temperature for 30 min, then 1.5 ml of erythrocyte lysis buffer was added, mixed and placed in dark place at room temperature for 10min to lyse erythrocytes. 2000g was taken to be centrifuged for 5 min and the supernatant was discarded, then added 1 ml 1×PBS into each tube, mixed and tested on the machine after centrifugation to remove the supernatant, and 10000 cells were gotten for each tube. According to the result of the detection, they were subdivided into 5 types:

Class A : CD3, CD4, CD8, CD4/CD8, NK and CIK were all normal

Class B: CD3, CD4, CD8 decreased, CD4/CD8 were normal; NK increased and CIK was normal

Class C: CD3 was normal, CD4 significantly decreased, CD8 significantly increased, CD4/CD8 significantly decreased, and NK and CIK were normal

Class D: CD3 and CD4 were normal, CD8 significantly decreased, CD4/CD8 significantly increased, and NK and CIK were normal

Class E: CD3 increased, CD4 was normal, CD8 significantly increased, CD4/CD8 significantly decreased, NK decreased, and CIK was normal

Statistically

SPSS16.0 was used for the statistical analysis, all data were presented as mean±standard deviation (x±s), chi-square test was used to the counting data, analysis of variance was used to measure data, LSD method was used to compare each group with the other, kaplan-meier was used to analyze maintaining time and Log-rank was used for the comparison between the two groups, and $P<0.05$ for the difference was judged to be statistically significant.

Results

Characteristics of the immune function of lung cancer patients

Before the treatment, according to characteristics of cells immune functions, 511 lung cancer patients were divided into Class A (147 cases, 28.8%), Class B (128 cases, 25.0%), Class C (142 cases, 27.8%), Class D (68 cases, 13.3%) and Class E (26 cases, 5.1%). Compared with normal people, cellular immune functions of Class A had no difference in all indicators ($P>0.05$). CD3, CD4 and CD8 of Class B were lower than those of normal people with the statistically significant difference ($P<0.05$), while there was no statistically significant difference in CD4/CD8, in addition, NK cells were higher than those of normal people with the statistically significant difference, and there was not statistically significant difference in CIK cells from that of normal people. For Class C, CD3, CD4 and CD4/CD8 were lower than normal with the statistically significant difference ($P<0.05$), CD8 and NK cells were higher than normal with statistically significant difference, and there was not statistically significant difference in CIK cells from normal people. For Class D, CD3 and CD8 were lower than normal with the statistically significant difference ($P<0.05$), CD4/CD8 was higher than normal with the statistically significant difference, and no statistically differences in CD4, NK and CIK cells from normal people. For Class E, CD4 and CD4/CD8 were lower than normal with the statistically significant difference ($P<0.05$), CD3 and CD8 were higher than normal with the statistically difference, and no statistically differences in NK and CIK cells from those of normal human (Table 1).

CIK treatment effects on the regulation of different immune dysfunction of lung cancer patients

After a course of CIK treatment, 31 cases of 67 patients in Class B improved (46.3%), the improvement rate was significantly higher than other Class, and it was the worst for Class D, only 8.0%, the difference was statistically significant ($P=0.000$). Class B, C and E improved significantly after the 2nd course of treatment, but only Class D significantly improved until the 3rd

Table 2. The Results of Adjusting Effects on Different Cellular Immune Dysfunction of the Lung Cancer Patients' Body by CIK Treatment

Groups	B		C		D		E		*P*
	N	Improvement cases (%)	N	Improvement cases (%)	N	Improvement cases (%)	N	Improvement cases (%)	
Control group	61	11(18.0)	70	6(8.6)	18	1(6.2)	6	0(0.0)	0.051
CIK cells treatment	67		72		50		20		
The first course		31(46.3)		14(19.4)		4(8.0)		5(25.0)	0
The second course		43(64.2)		38(52.8)		18(36.0)		9(45.0)	0.023
The third course		51(76.1)		46(63.9)		31(62.0)		11(55.0)	0.2
The forth course		52(77.6)		49(68.1)		36(72.0)		13(65.0)	0.557
The fifth course		53(79.1)		51(70.8)		38(76.0)		14(70.0)	0.675
The total effective cases		53(79.1)		51(70.8)		38(76.0)		14(70.0)	0.675

Table 3. Maintaining Time of Normal Status of Cellular Immune Function in Class B Lung Cancer Patients

Course	The rate to maintain normal				median time	95%CI
	1 month	3 months	6 months	12 months	(months)	
Class B lung cancer patients						
1st	0.903	0.742	0.161	0	4.1	3.23-4.97
2nd	0.93	0.86	0.465	0.023	5.8	4.64-6.96
3rd	0.932	0.902	0.667	0.039	6.3	6.00-6.60
4th	1	0.942	0.798	0.115	9.8	8.59-11.01
5th	1	0.962	0.83	0.151	10.4	9.76-11.04
Class C lung cancer patients						
1st	0.786	0.214	0	0	2.2	1.83-2.57
2nd	0.895	0.658	0.237	0	3.6	2.54-4.66
3rd	0.913	0.891	0.543	0.022	6.2	4.68-7.72
4th	0.98	0.898	0.714	0.041	8.2	7.97-8.43
5th	1	0.98	0.745	0.078	8.4	7.86-8.94
Class D lung cancer patients						
1st	0.5	0	0	0	1	0.61-1.39
2nd	0.833	0.444	0	0	2.6	0.73-4.47
3rd	0.903	0.71	0.355	0	5.2	4.00-6.40
4th	1	0.917	0.806	0.056	8.8	8.41-9.19
5th	1	0.974	0.842	0.184	9.8	9.20-10.4
Class E lung cancer patients						
1st	0.6	0	0	0	1.2	0.77-1.63
2nd	0.778	0.333	0	0	2.6	2.31-2.89
3rd	0.818	0.636	0.182	0	4.5	2.88-6.12
4th	0.923	0.769	0.538	0.077	6.6	4.02-9.19
5th	1	0.929	0.643	0.143	7.9	6.25-9.55

course of treatment. Improvement rates of 4 groups at the end of 5 courses were 79.1%, 70.8%, 76.0% and 70.0%, respectively, all significant, and the difference was not statistically significant (*P*=0.675). For Class B, after the 1st course of treatment, the improvement rate was 46.3% and 64.2% after the 2nd course, the difference between them was statistically significant (*P*=0.001), and after the 3rd course, the situation stabilized without statistical significance (*P*=0.131) compared with the 4th course. For Class C, there was no significant improvement in the 1st course with the improvement rate of 19.4%, while there was a significant improvement in the 2nd course, with the improvement rate of 52.8%, the difference was statistically significant (*P*=0.000), and after the 3rd course, the situation stabilized without statistical significance (*P*=0.176) compared with the 4th course. For Class D, in the 1st and 2nd course, the improvements were not obvious, until the 3rd course, the improvement was significant with rate of 62.0%, the difference was statistical (*P*=0.009), and there was not obvious change in the 4th and 5th course, comparing with the 3rd course, the difference was not statistically significant (*P*=0.288). Class E improved significantly after the 2nd course, the improvement rate was 45.0%, and the situation became stabilized after the 3rd and 4th course (Table 2).

Maintaining time of normal status of cellular immune function in lung cancer patients

Maintaining time of normal status of cellular immune function in Class B lung cancer patients: For Class B patients, whose immune function restored normal at the end of the 1st course, the median time of keeping normal immune function was 4.1 months (3.23-4.97), after the 2nd course, it was 5.8 months (4.64-6.96), 6.3 months (6.00-6.60) after the 3rd course, 9.8 months (8.59-11.01) after the 4th course and 10.4 months (9.76-11.04) in the end of 5 courses. The time of maintaining normal all extended after different courses, there were no statistically significant difference (*P*=0.534) in remaining time between the 2nd and the 3rd course, as well as the 4th and the 5th, *P*=0.000 (Table 3 and Figure 1).

Maintaining time of normal status of cellular immune function in Class C lung cancer patients: For Class C patients, whose immune function restored normal at the end of the 1st course, the median time of keeping immune function normal was 2.2 months (1.83-2.57), after the 2nd course, it was 3.6months (2.54-4.66), and 6.2 months (4.68-7.72) after the 3rd course, with obvious increasing and statistically significant difference when compared with the 1st and 2nd (both *P*=0.000). The median time was 8.2 months (7.97-8.43) after the 4th course, with statistically significant difference when compared with the 3rd (P=0.018) and 8.4 months (7.86-8.94) in the end of 5 courses without statistically difference (*P*=0.626) (Table 3 and Figure 1).

Maintaining time of normal status of cellular immune function in Class D lung cancer patients: For Class D patients, whose immune function restored normal after the end of the 1st course, the median time of keeping immune function normal was 1.0 month (0.61-1.39), after the 2nd course, it was 2.6 months (0.73-4.47) with more slowly rising, and 6.2 months (4.68-7.72) after the 3rd course with obvious increasing. The median time was 8.8 months (8.41-9.19) after the 4th course and 9.8 months (9.20-10.4) in the end of 5 courses. There were statistically significant

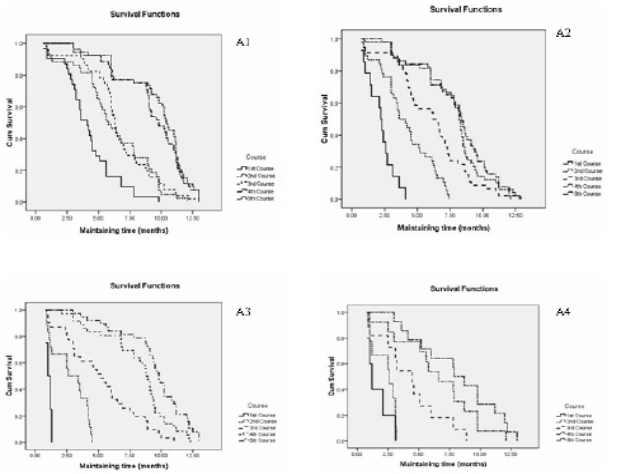

Figure 1. Maintaining Time of Normal Status of Cellular Immune Function in Lung Cancer Patients

differences, when comparing with the medium time of each courses (Table 3 and Figure 1).

Maintaining time of normal status of cellular immune function in Class E lung cancer patients: For Class E patients, whose immune function restored normal at the end of the 1st course, the median time of keeping immune function normal was 1.2 months (0.77-1.63), after the 2nd course, it was 2.6 months (0.73-4.47) without statistically significant difference (P=0.272) compared with the 1st. The median time was 4.5 months (2.88-6.12) after the 3rd course with statistically significant difference (P=0.012) when compared with the 2nd, and 6.6 months (4.02-9.19) after the 4th course with statistically significant difference (P=0.049) when compared with the 3rd. The median time was 7.9 months (6.25-9.55) in the end of 5 courses without statistically significant difference when compared with other courses (P=0.170) (Table 3 and Figure 1).

Comparison of maintaining time of restoring immune function after the same treatment course in different Class lung cancer patients: From Table 3, it could be seen that at the end of the 1st course, the maintaining time of Class B was 4.1 months (3.23-4.97), longer than other Class with statistically significance differences (P=0.000 for all), and the maintaining time of D and E were worst without statistically significance difference (P=0.347) between them but statistically significance differences (P=0.007 and 0.000, respectively) when compared with C. After the 2nd course, it was still longer for Class B than other Class with statistically significant differences (P=0.000 for all), D and E were poor without statistical significance (P=0.066), and C was different from D but not from E with P as 0.004 and 0.076, respectively. After the 3rd course, the maintaining time of B, C, D and E were significantly prolonged, but there were only statistically significant differences between B and D or E with P as 0.05 and 0.007, respectively. The difference was not statistically significant in other comparisons with P all greater than 0.05. After 5 courses, B and D maintained the longest without statistical significance (P=0.589) between them, while C and E maintained a short period without statistically significant difference (P=0.444) between

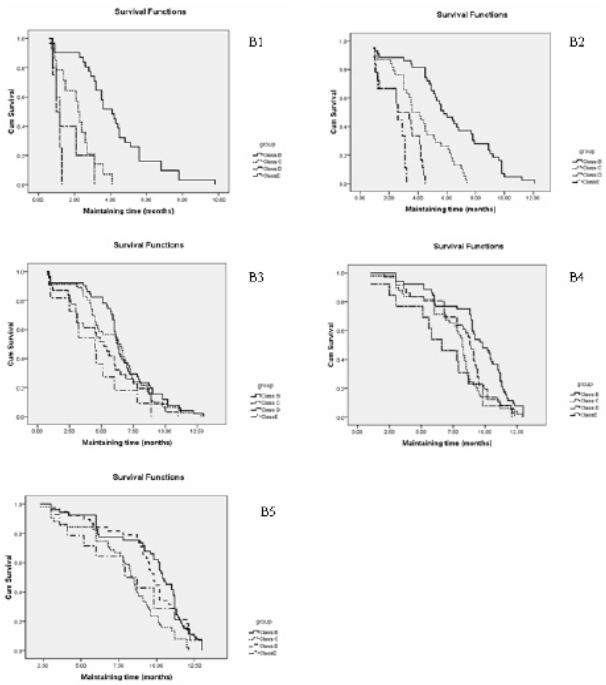

Figure 2. Comparison of Maintaining Time of Restoring Immune Function after the Same Treatment Course in Different Class Lung Cancer Patients

them, but with statistically significant difference (P <0.01) when compared with B and D (Figure 2).

Discussion

The incidence and mortality rates of lung cancer are in the first place of malignancy in the world, of which more than 80% are NSCLC, characterized by high incidence, high mortality and low survival (Levina et al., 2010). Standardized radiotherapy could kill the majority of tumor cells in a certain extent, but because of such factors as severe toxicity, resistant cells, the existence of cancer stem cells and anti-radiation cell, low immunity of advanced tumors patients and other factors, the implementation and benefit of the treatment program are greatly hampered (Levina et al., 2010; Baas et al., 2011; Hsu et al., 2011). Therefore, it is received widespread attention to explore and research new methods of treatment.

There are CD system antigens in normal T cell surface. CD3+T are all mature T cells, CD4+T are assistant T lymphocyte, and CD8+T are inhibitory T lymphocytes. The proportion of these cells in the human body is constant to maintain its optimum state of equilibrium, in order to achieve the cellular immune surveillance (Shepherd et al., 2011). The main role of CD4 T cells as the assistant T lymphocytes is to identify exogenous antigen peptide, presented by MHC I molecules, differentiating to Th cells after the activation (Bordón et al., 2011). The function of CD8 T cell is to inhibit the activation stage of immune response, and its target cells are antigen-specific Th cells and B cells. Human tumor-specific cytotoxic T lymphocytes are all CD8 T cells, the changes of which could lead to tumor-specific anti-tumor effect weakening. Studies and clinical researches both showed that the CD4+/CD8+ might well reflect the balance of the host

immune regulation, and its ratio-decreasing indicated the reducing of patients' immune function and increasing of opportunities of malignant tumor occurrence (Das et al., 2007). NK cells are effector cells, playing the role in early stage of tumor, requiring no specific antibodies or pre-sensitized lymphocytes, it could quickly be activated to suppress and destruct a variety of tumor cells, and more lethal after affected by lymphokines (Kuss et al., 2004).

Immune functions of lung cancer patients are low, so the body could not effectively carry out the immune defense reaction, which is an important factor leading to tumor cell immune escaping and postoperative metastasis recurrence (Hongeng et al., 2003; Shi et al., 2004; Sievers et al., 2004; Wang et al., 2006; Méndez et al., 2007; Wongkajornsilp et al., 2009). In this study, there were different types of immune function disorders of peripheral blood cells in lung cancer patients, which were distributed to 147 cases Class A (28.8%), 128 cases Class B (25.0%), 142 cases Class C (27.8%), 68 cases Class D (13.3%) and 26 cases Class E (5.1%) among 511 lung cancer patients according to the cells immune characteristics before treatment. Compared with normal people, cellular immune functions of Class A had no difference ($P > 0.05$). CD3, CD4 and CD8 of Class B were lower than those of normal people with statistically significant difference ($P<0.05$), but no statistically significant difference in the ratio of CD4/CD8, and NK cells were higher than normal people with statistically significant difference, and the difference in CIK cells was not statistically significant. For Class C, CD3, CD4 and CD4/CD8 were lower than those of normal people with the statistically significant difference ($P<0.05$), CD8 and NK cells were higher than normal with statistically significant difference, and there was no statistically significant difference in CIK cells when compared with normal. For Class D, CD3 and CD8 were lower than normal with statistically significant difference ($P<0.05$), CD4/CD8 was higher with statistically significant difference, and there were not statistically significant difference in CD4, NK and CIK cells when compared with normal people. For Class E, CD4 and CD4/CD8 were lower than those of normal person with statistically significant difference ($P<0.05$), CD3 and CD8 were higher than those of normal with statistically significant difference, and there were not statistically significant differences in NK and CIK cells when compared with normal people.

Treatment of CIK is the new strategy for tumor adoptive immunotherapy developed recent years (Höltl et al., 1999; Blattman and Greenberg, 2004). CIK cells are heterogeneous cell groups, including two subsets CD3-CD16+CD56+ and CD3+CD16+CD56+, which are obtained by people PMBC co-incubation in vitro with a variety of cytokines, such as CD3McAb, IL-2, IFN-γfor some time, the main effector cells are CD3+CD56+ cells, known as effector cells of a tumor biological treatment with the strongest cytotoxic activity. The CIK cells could kill tumor cells through three ways (Zhang et al., 2007), the 1st way, CIK cells could recognize tumor cell surface ligands by Mcli20 and form proteins and particle enzymes through perforation to dissolve cells and directly kill tumor cells. the 2nd way, a variety of cytokines (IL-2, IFN-γ, TNF, etc.) are secreted by the CIK cells into the body, could not only directly inhibit tumor cells, but also indirectly kill them by regulating the body's immune system, at the same time, enhance the anti-tumor function of T cells. Finally, the 3rd way, CIK cells could induce apoptosis of tumor cells through the expression of FasL, and express anti-apoptotic genes (Bcl-2, Bcl-xl, SurvIvin) to resist the counterproductive effect of FasL-positive tumor cells, which would make CIK cells be able to tolerate the apoptosis induced by tumor cell, which would express apoptosis-related factor ligands, and thus play a long-lasting anti-tumor effect (Xu et al., 2010).

This study showed that the immune functions of lung cancer patients with different cell immune dysfunctions achieved improvements after CIK cell therapy, but the needs for the treatment courses of CIK were different. In this study, after 5 courses, immune function improvement rates could all be over 70%, consistent with the report of Li et al. (2012) on kidney cancer immune function improvement. This study suggested that the improvements of immune function by CIK cells were affected by two factors, the first factor was the situation of immune function of lung cancer patients prior to the treatment, different immune functional status would react differently to CIK therapy. The second factor was the number of CIK cells. Only a treatment course of CIK could not improve the immune function of lung cancer patients, while several courses of treatment could achieve a stable effect. Among of the five types patients, Class B needed the fewest courses, only 1-2 could achieve stable effects, while Class C needed 2-3 courses, 3-4 courses for Class D with the worst effect in the end of the 1st course, and 2-3 courses for E, thus it was prompted that CIK cell therapy program should be reasonably arranged according to functional status of lung cancer patients after the CIK treatment.

To maintain and try to extend the time of the anti-tumor immune is equally important with stimulating the body's anti-tumor immunity (Méndez et al., 2007; Wongkajornsilp et al., s2009). In present, in CIK cell therapy, there is more controversy on the questions of the number of cells needed to be infused and how long the body's anti-tumor immune function could last after the infusion. In this study, the results of immune function test of patients showed that the recovery of immune function of lung cancer patients could last for some time after CIK cell therapy, but the durations were different according to different courses and different types of immune disorders. This prompted that the CIK treatment cycle needed to be designed according to the immune function of the patient's condition and the treatment times, which would enable patients to last long-term maintenance of normal immune function and achieve long-term fighting against cancer and longer survival time.

This study suggested that at the end of the 1st course of treatment, the maintaining time of Class B was longer, being 4.1 months (3.23-4.97), suggesting that patients needed another course of treatment at the 4th month from the end of the 1st course, while it were 2.2 months (1.83-2.57), 1.0 (0.61-1.39) and 1.2 months (0.77-1.63) for Class C, D and E, respectively, suggesting that these patients needed another course of treatment at the 1st or 2nd

month from the end of the 1st course, it was significantly different from the traditional CIK cell treatment interval, in which the current interval is 3 months after the 1st course of treatment before the next course. At the end of the 2nd course, the median time for the duration of the 4 types were 5.8 month (4.64-6.96), 3.6 months (2.54-4.66), 2.6 month (0.73-4.47) and 2.6 month (2.31-2.89), respectively, significantly different from the traditional CIK cell treatment interval, and the current interval is 6 months after the 2nd course before the next course starts. At the end of five courses, the duration of the 4 types were significantly prolonged, but did not reach 1 year. This prompted the appropriate period of treatment needed to be given according to the changes in immune functions during CIK cell treatment.

In conclusion, CIK cells could improve the immune function of lung cancer patients, but the quality of improvement was associated with the immune function conditions before treatment and the treatment courses. The improvements of cellular immune function of lung cancer patients were able to remain for some time, but the length of the maintaining time was still associated with the immune function conditions before treatment and the treatment courses. It needed to select different time intervals according to different situations during CIK cell treatment.

References

Baas P, Belderbos J SA, van den Heuvel M (2011). Chemoradiation therapy in n on small cell lung cancer. *Curr Opin Oncol*, **23**, 140-9.

Blattman JN, Greenberg PD (2004). Cancer immunotherapy: a treatment for the masses. *Science*, **305**, 200-5.

Bordón E, Henríquez-Hernández LA, Lara PC (2011). Role of CD4 and CD8 T-lymphocytes, B-lymphocytes and Natural Killer cells in the prediction of radiation-induced late toxicity in cervical cancer patients. *Int J Radiat Biol*, **87**, 424-31.

Das S, Karim S, Datta Ray C (2007). Peripheral blood lymphocyte subpopulations in patients with cervical cancer. *Int J Gynaecol Obstet*, **98**, 143-6.

Höltl L, Rieser C, Papesh C, et al (1999). Cellular and humoral immune responses in patients with metastatic renal cell carcinoma after vaccination with antigen pulsed dendntic cell. *J Urol*, **161**, 777-82.

Hongeng S, Petvises S, Worapongpaiboon S, et al (2003). Generation of CD3+CD56+ cytokine induced killer cells and their invitro cytotoxicity against pediatric cancer cells. *Int J Hematol*, **77**, 175-9.

Hsu HS, Huang PI, Chang YL, et al (2011). Cucurbitacin i inhibits tumorigenic ability and enhances radiochemosensitivity in nonsmall cell lung cancer-derived CD133-positive cells. *Cancer*, **117**, 2970-85.

Kim YJ, Lim J, Kang JS, et al (2010). Adoptive immunotherapy of human gastric cancer with ex vivo expanded T cells. *Arch Pharm Res*, **33**, 1789-95.

Kuss I, Hathway B, Ferris RL, et al (2004). Decreased absolute counts of T lymphocyte subsets and their relation to disease in squamous cell carcinoma of the head and neck. *Clin Cancer Res*, **10**, 3755-62.

Levina V, Marrangoni A, Wan g T, et al (2010). Elimination of human lung cancer stem cells through targeting of the stem cell factor-c-kit autocrine signaling loop. *Cancer Res*, **70**, 338-46.

Li R, Wang C, Liu L, et al (2012). Autologous cytokine-induced killer cell immunotherapy in lung cancer: a phase II clinical study. *Cancer Immunol Immunother*, **61**, 2125-33.

Méndez R, Ruiz-Cabello F, Rodríguez T, et al(2007). Identification of different tumor escape mechanisms in several metastases from a melanoma patient undergoing immunotherap. *Cancer Immunol Immunother*, **56**, 88-94.

Shepherd FA, Douillard JY and Blumenschein GR Jr (2011). Immunotherapy for non-small cell lung cancer: novel approaches to improve patient outcome. *J Thorac Oncol*, **6**, 1763-73.

Shi M, Zhang B, Tang ZR, et al (2004). Autologous cytokine induced killer(CIK)cell therapy in clinical trial phaseIissafe in patients with primary hepatocellular carcinoma. *World J Gastroenterol*, **10**, 1146-51.

Sievers E, Albers P, Schmidt-Wolf IG , et al (2004). Telomerase pulsed dendritic cells for immunotherapy for renal cell carcinoma. *J Urol*, **171**, 114-9.

Urbanska K, Lanitis E, Poussin M, et al (2012). A universal strategy for adoptive immunotherapy of cancer through use of a novel T-cell antigen receptor. Cancer Res, 72 ,1844-52.

Wang H, Zhou FJ, Wang QJ (2006). Efficacy of autologous renal tumor cell lysate- loaded dendritic cell vaccine in combination with cytokine- induced killer cells on advanced renal cell carcinoma- - a report of ten cases. *Ai Zheng*, **25**, 625-30.

Wongkajornsilp A, Somchitprasert T, Butraporn R, et al (2009). Human cytokine- induced killer cells specifically infiltrated and etarded the growth of the inoculated human cholangiocarcinoma cells in SCID mice. *Cancer Invest*, **27**, 140-8.

Wu F, Wang ZB, Lu P, et al (2004). Activated anti-tumor immunity in cancer patients after high intensity focused ultrasound ablation. *Ultrasound Med Biol*, **30**, 1217-22.

Xu Y, Liu LJ, Gao JM, et al (2010). Specific Anti- tumor Biological Activity of DCIK Cells. *China Modern Doctor*, **27**, 360-2

Zhang H, Zhao Q, Zuo LF, et al (2007). The functional mechanism of CIK cell on ovarian carcinoma cell lines SKOV3/CDDP. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*, **23**, 1167-9.