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Original Article

Effect of immunotherapy on the immune function and survival of patients after colon cancer surgery

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Abstract: To investigate the effect of DC-CIK treatment on the immune function and survival of patients with colon cancer. 102 colon cancer patients were randomly divided into control group and observation group. The control group was treated conventionally, while the observation group was treated with DC-CIK combined with chemotherapy. After the treatment, flow cytometry detected the proportions of peripheral blood $CD4^+$, $CD8^+$, $CD16^+CD56^+$, $CD4^+CD25^+$ and the ratio of $CD4^+/CD8^+$ before and after treatment, and the changes of immune functions of both groups before and after treatment were compared; ELISA was used to detect the changes of the peripheral Th1/Th2 cytokine levels of both groups before and after treatment; the cumulative survival rates of both groups after treatment were compared. After treatment, the observation group has an obviously improved lymphocyte levels than the control group, and the difference is of statistical significance ($P<0.05$); after treatment, the level of peripheral serum Th1 cytokines (IL-2 and IFN-γ) of the observation group significantly increased as compared with that before treatment and that of the control group after treatment ($P<0.05$), but Th2 cytokine (IL-6 and IL-10) levels decreased remarkably after treatment as compared those before treatment and those of control group after treatment ($P<0.05$); one-, two- and three-year cumulative survival rates are 86.27%, 72.55% and 56.86% respectively; one-, two- and three-year cumulative survival rates of the observation group are 92.16%, 84.31% and 70.59% respectively; there was significantly difference in accumulative survival rates between two groups ($P<0.05$). In conclusion, DC-CIK combined with chemotherapy can effectively adjust the proportion of lymphocyte and Th1/Th2 balance in colon cancer patients, markedly improve immune function and increase survival rate.

Keywords: DC-CIK, chemotherapy, colon cancer, Th1/Th2 balance

Introduction

Colon cancer is one of the common malignant gastrointestinal cancers and found in colon. Its incidence ranks the third among gastrointestinal tumors with high mortality. Clinical observation indicates that the survival rate of colon cancer patients in IV period is only 8.1% [1]. Until now, surgery is still the major method of treating colon cancer. Radiochemotherapy is a common adjuvant method of treating many malignant tumors, including colon cancer. Radiochemotherapy can reduce tumor burden and thus relieve the immunologic suppression directly induced by tumor cells; it also may damage organism's immune system, marrow and other hemopoietic systems, inhibit the production of immune cells, and also arouse patients' gastrointestinal reactions, resulting in poor appetite, nausea, vomiting and inadequate nutritional intake. All lead to the decrease of patients' immune functions and thus affecting organism's resistance to cancer cells [2-4]. Therefore, it is urgent to seek more effective treatment methods for colon cancer patients, especially for those who cannot undergo surgery at medium and advanced stages and those who cannot tolerate radiochemotherapy. Adoptive immunotherapy mediated by cells comes into being. It has become one of the four methods of treating tumors, as the other three ones are surgery, radiation therapy and chemotherapy [5, 6]. It is effective in promoting the reconstruction of patients' immune system, eliminating residual focus and cleaning bone marrow. Cytokine induced killer (CIK) cell is heterogeneity cell which kills many tumors and is induced by various cytokines inducing

peripheral blood mononuclear cell (PBMC), or umbilical cord blood mononuclear cell in vitro, with the powerful tumor-killing activity of T-lymphocyte and the non-MHC limited tumor-killing advantage of NK cells. Dendritic cells (DC), the most powerful specific antigen -presenting cells (APC) currently, can induce antigen-specific immune response and effective excite T-cell response and thus resist the immune escape mechanism of tumor cells [7, 8]. The combination of DC-CIK cells has the advantages of antigens' identification, presenting and effect cells' immune cytotoxicity, which makes sure the specificity and effectiveness of immune therapy. In this research, DC-CIK combined with chemotherapy was taken to treat colon cancer patients so as to observe the effect of DC-CIK on patients' immune functions and survival.

Subjects and methods

General data

A total of 102 cases of colon cancer treated in our hospital from January, 2010 to January, 2013 were selected. Inclusion criteria: ① all patients were confirmed to be with colon cancers by post-pathologically examination, and at II and III stages according to the TNM staging of colon cancer standard made by Union for International Cancer Control (UICC) in 1997; ② no severe complications like intestinal obstruction or massive hemorrhage; ③ Karnofsky score KPS≥70; ④ no severe diseases related to heart, lung, liver or kidney; ⑤ patients did not undergo other treatment in recent one month; ⑥ patients had signed the informed consent. The patients were randomly divided into control group and observation group. In control group, there are a total of 51 cases, including 27 males and 24 females, aging from 42 to 74 and the median age is 54.9 years; clinical staging: 25 cases are at stage II and 26 cases at stage III. In the observation group, there are a total of 51 cases, including 28 males and 23 females, aging from 45 to 75 and the median age is 56.7 years; clinical staging: 24 cases are at stage II and 27 cases at stage III. There is no statistical significance in the differences in gender, age and clinical stage of both groups. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all participants.

DC-CIK cell preparation

Before chemotherapy, 60 ml of patients' peripheral blood mononuclear cells (PBMC) was collected with blood cell separator (Fresenius Kabi, Bad Homburg, Germany) and centrifugated at 2000 rpm/min for 15 min to get plasma which was stored at -20°C; cells were deposited and resuspended with PBS and then added into the upper separation liquid of lymphocytes (Haoyang Biotechnology Co., Tianjin, China) in the proportion of 1:2; after centrifugation, the white film layer was taken to get mononuclear cells. After resuspending cells with 50 ml AIMV serum-free medium, 5 ml suspension was taken and cell concentration was adjusted to be $1\text{-}2\times10^7$/ml with AIMV serum-free medium; add it into the two 6-well plates which were pre-added with 1 ml serum-free medium per well, 1 ml/well; the plates were cultured for 2 h at 37°C, 5% CO_2, and the non-adherent cells were sucked out, while the adherent cells were added into the serum-free medium containing GM-CSF (1000 U/ml) and IL-4 (500 U/ml) for DC culture; every three days, the complete medium with the previous factors were changed; on the 7^{th} day, TNF-α (1000 U/ml) was added for accelerating maturing; in the remaining 45 ml suspension cells, serum-free medium containing INF-γ (1000 U/ml) were added for CIK culture; on the second day, IL-1α (100 U/ml), IL-2 (500 U/ml) and CD3 monoclonal antibody (50 ng/ml) were added, and complete medium containing previous cytokines were changed every three days; on the 8^{th} day, the matured DC was collected and mixed with CIK in the proportion of 1:5 to 1:10; later they were cultured in 1.8 L cell culture bags, which was DC-CIK; after 8 days, cells were collected for retransfusion, and human serum albumin with 1% of mass fraction was added; it was injected into 0.9% sodium chloride injection with injector for retransfusion, once every day, 5 times together, cell number $>2\times10^9$ every time. All cells would not be retransfused until they were detected to be bacteria, fungi, and endotoxin negative.

Treatment methods

The two groups were conducted the same therapy: FOLFOX4 therapy: L-OHP 85 mg/m^2, intravenous infusion 2 h, d1; CF 200 mg/m^2 intrave-

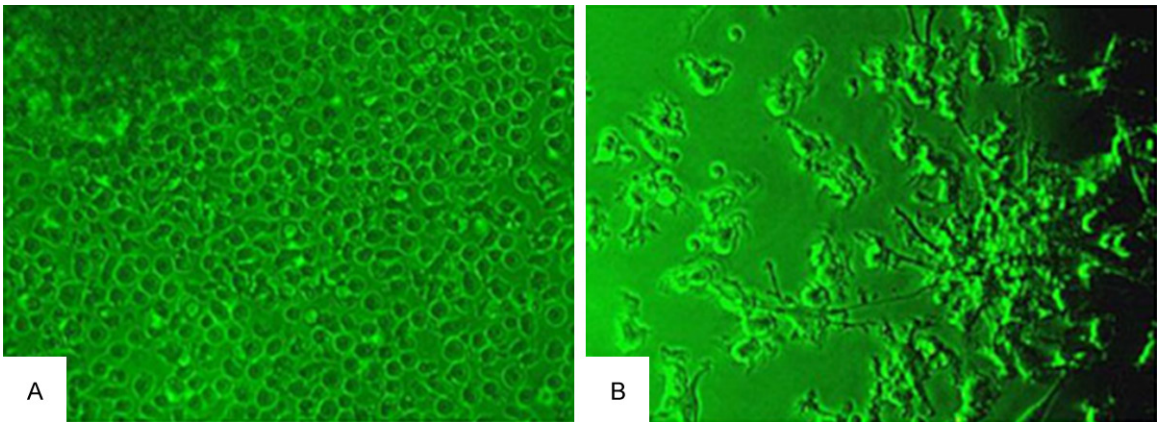
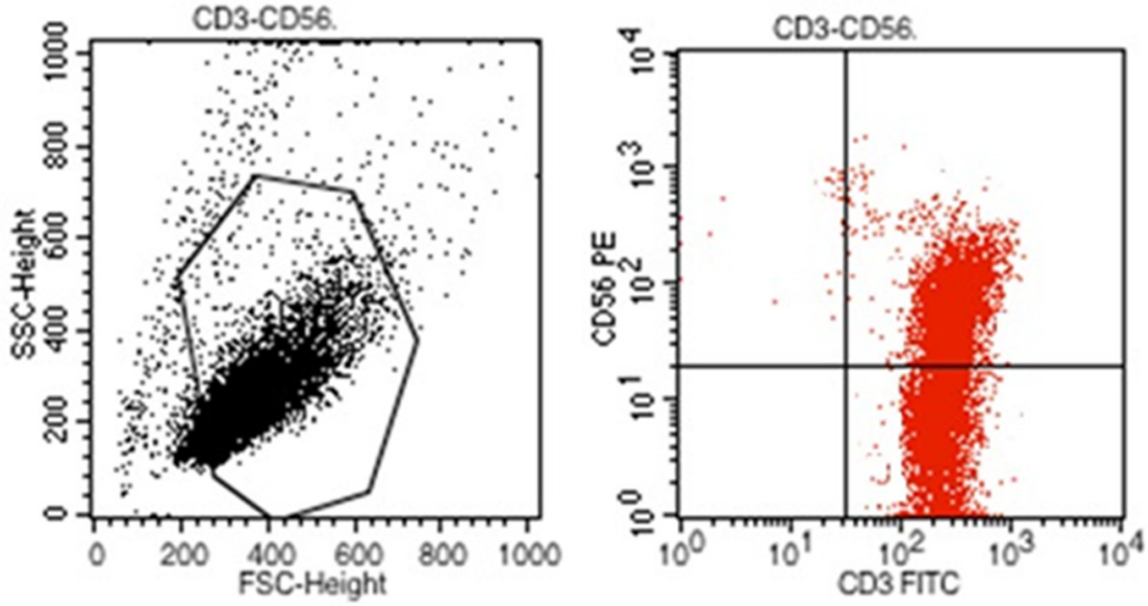


Figure 1. A. CIK cultured on the 8th day under 200× microscope; B. DC after maturing acceleration under 400× microscope.



Region	Events	% Gated	% Total
R1	8329	83.29	83.29

Quad	Events	% Gated	% Total
UL	119	1.35	1.19
UR	4379	49.63	43.79
LL	2	0.02	0.02
LR	4329	49.13	43.29

Figure 2. $CD3^+CD56^+$ double-positive rate of DC-CIK cells of one patient cultured on 16th day.

nous infusion 2 h, d1-2; 5-Fu 400 mg/m^2 intravenous infusion 2, d1-2; 5-Fu 600 mg/m^2, continuous intravenous infusion 22 h, d1-2; 12 d is a cycle. Meanwhile, PMBC of observation group was collected 1 day before chemotherapy for DC-CIK culture; after chemotherapy, DC-CIK was retransfused. Three months later, both groups underwent the second course of treatment. There were a total of 3 treatment courses.

Index observation and effect determination

2 ml of peripheral blood was extracted before treatment and two weeks after treatment. Flow cytometry (BD Biosciences, New Jersey,

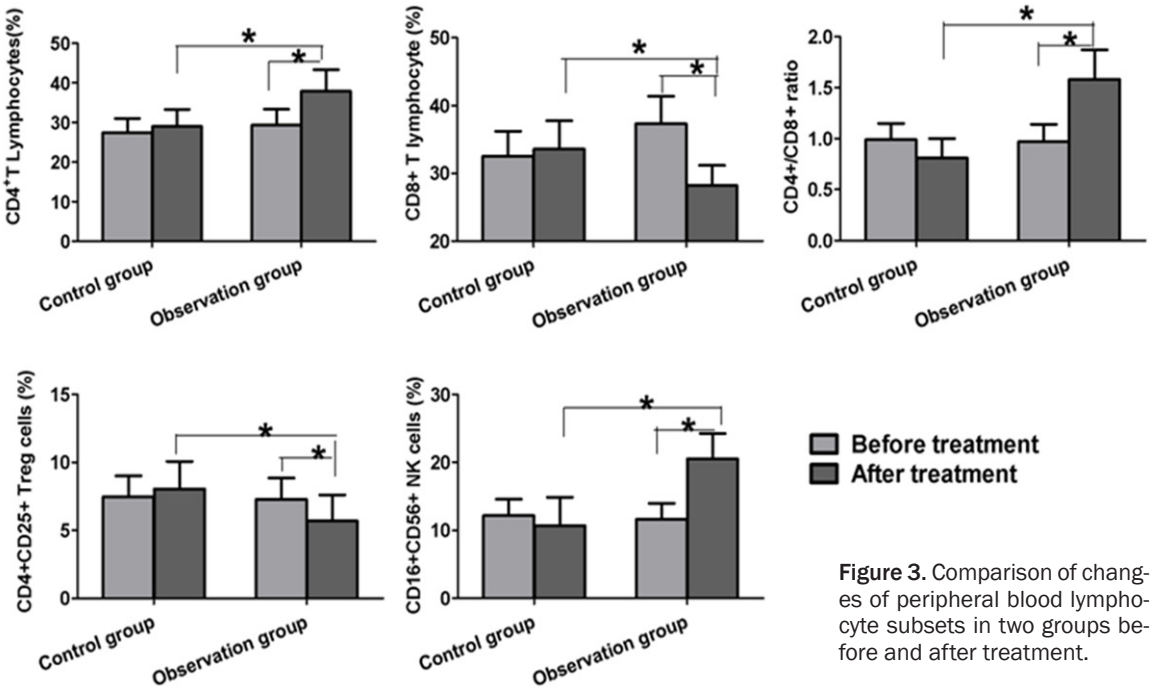


Figure 3. Comparison of changes of peripheral blood lymphocyte subsets in two groups before and after treatment.

USA) was used to detect the proportions of peripheral blood $CD4^+$, $CD8^+$, $CD16^+CD56^+$, $CD4^+CD25^+$ and the ratio of $CD4^+/CD8^+$; 3 ml of patients' fast venous blood was taken and centrifugated for serum, and ELISA (R&D Systems Inc, Minneapolis, MN, USA) was employed to detect the levels of serum Th1 cytokines (IL-2 and IFN-γ) and Th2 cytokines (IL-6 and IL-10); the cumulative survival rates of patients were analyzed.

Statistical analysis

All data were analyzed with SPSS17.0 software (SPSS Inc, Chicago, IL, USA), and measured data was expressed by $\bar{x} \pm s$ and measured information tested by t-test; count data was checked with Chi-square test; the comparison of cumulative survival rates was tested with Kaplan-Meier method and Log-Rank. $P<0.05$ was considered statistically significant.

Results

Cell morphology and phenotype

The randomly selected DC cells and CIK cells which had been cultured for eight days from a patient were observed under 200× microscope to get their morphology (**Figure 1**); the randomly selected DC-CIK cells which were retransfused on the first day from one patient was analyzed with flow cytometry to detect its $CD3^+$ $CD56^+$ double-positive rate (**Figure 2**).

Changes of peripheral blood lymphocyte subsets of both groups before and after treatment

As shown in **Figure 3**, the comparison of peripheral blood $CD4^+$, $CD8^+$, $CD16^+CD56^+$, $CD4^+CD25^+$ cell proportions and $CD4^+/CD8^+$ ratio of both groups before treatment is of no statistical significance ($P>0.05$). In the control group, no obvious difference exists in $CD4^+$, $CD8^+$, $CD4^+/CD8^+$, $CD16^+CD56^+$ and $CD4^+CD25^+$ cell proportions before and after treatment ($P>0.05$). In the observation group, patients' peripheral blood $CD4^+$, $CD16^+CD56^+$ cell proportions and $CD4^+/CD8^+$ ratio after treatment are higher than those after treatment, while $CD8^+$ T cell and $CD4^+CD25^+$ regulatory T cell proportions are lower than those before treatment ($P<0.05$).

Th1/Th2 cytokine changes of both groups before and after treatment

No statistical significance is found in the difference in peripheral blood Th1/Th2 cytokine levels of both groups ($P>0.05$) (**Figure 4**). After treatment, both groups have higher peripheral blood cytokine IL-2 and IFN-γ levels than those before treatment, and the change is more marked in observation group ($P<0.05$); both

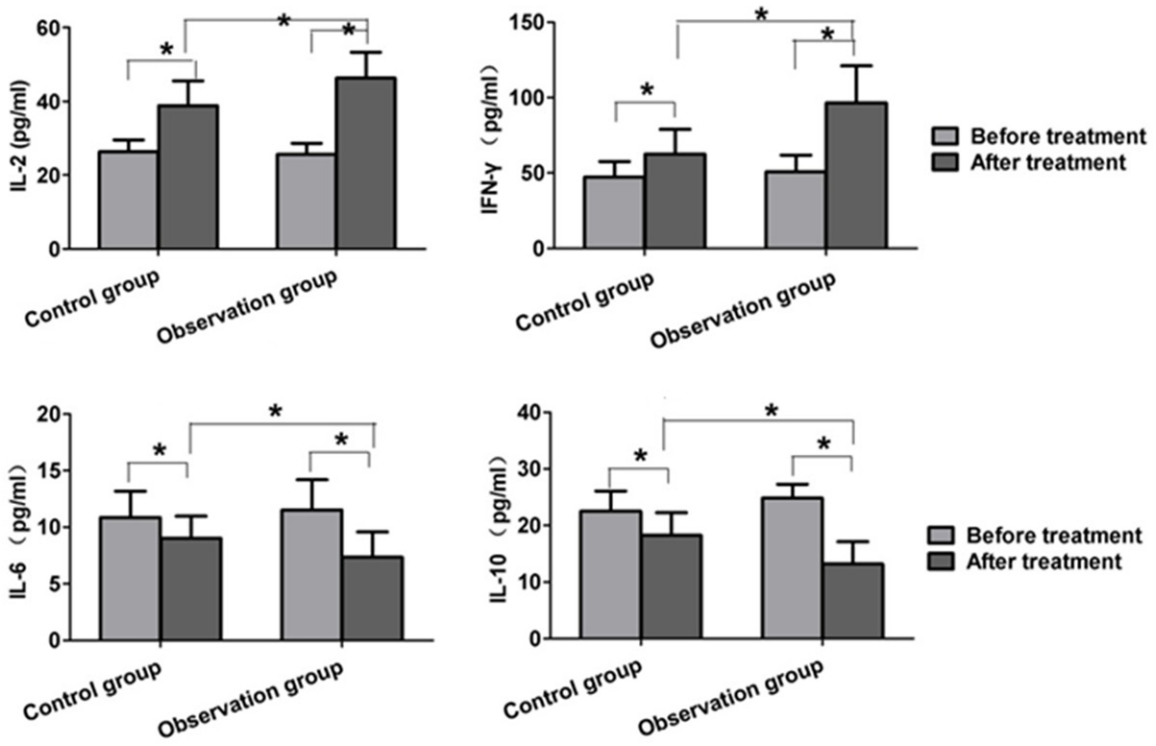


Figure 4. Comparison of Th1/Th2 cytokines in two groups.

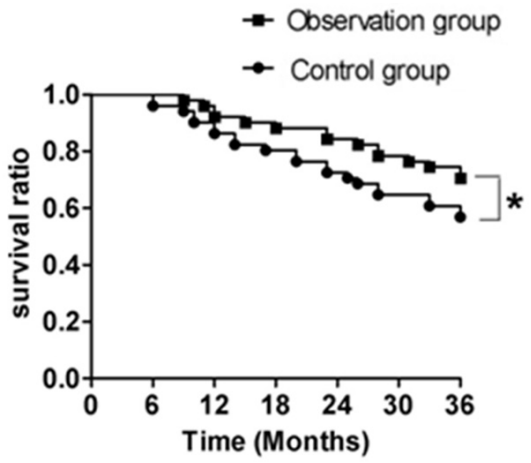


Figure 5. Comparison of cumulative survival rates after treatment in two groups.

groups' cytokine IL-6 and Il-10 levels are lower than those before treatment, and the change is more significant in observation group ($P<0.05$).

Comparison of cumulative survival rates of both groups

After the treatment of both groups, the follow-up lasted 3-36 months. There are 22 cases of death in control group and 15 cases in observation group. Kaplan-Meier curve shows that the one-, two- and three-year cumulative survival rates of control group are respectively 86.27% (44 cases), 72.55% (37 cases) and 56.86% (29 cases), while those of observation group are respectively 92.16% (47 cases), 84.31% (43 cases) and 70.59% (36 cases). Log-Rank test indicates that the difference of cumulative survival rates of the two groups is statistically significant ($P<0.05$) (**Figure 5**).

Discussion

As one of malignant gastrointestinal cancers, colon cancer has an increasing incidence and mortality, and surgery and post-operative adjuvant therapy can slow down the disease development [9]. Post-operative chemotherapy is important in controlling, eliminating residual and micro metastases. However, due to the reduced immune functions of tumor patients, multi-cycle chemotherapy leads to patients' intolerance, and causes further injury of organisms' immune function [10]. The cell immunotherapy based on CIK and DC solves the problem.

DC-CIK cell therapy is a commonly used cell immunotherapy in clinics. As is demonstrated by many researches, the co-culture of DC-CIK cells increases DC's antigen presenting and stimulation of organism's immune response, and also gives a rise to the proliferation activity and cytotoxicity of CIK [11]. DC-DIK combined with radiochemotherapy has become the first clinical choice of post-operative or non-surgery treatment of cancers. Wang et al. [12] had a systematic review of published theses. They employed the data of random or fixed effect model to analyze the effect of DC-DIK combined with chemotherapy on Chinese colon cancers. The overall analysis indicates that, treated by DC-DIK combined with chemotherapy, patients had a significantly increased one-, two- and three-year cumulative survival rates and $CD4^+$ T cells, which manifests that DC-DIK combined with chemotherapy is more effective on prolonging colon cancer patients' survival and increasing immune response than chemotherapy alone. In this research, after undergoing DC-DIK combined with chemotherapy, patients had significantly higher one- and two-year survival rates than chemotherapy group, which further verifies the conclusion of Wang et al.

$CD8^+$ cell in patients' peripheral blood mainly inhibits T cells and help inhibiting the activity of T helper cells (Th), and thus indirectly suppress the differentiation of B-cells and the killing ability of T-cell cytotoxicity (Tc). It can negatively regulate humoral immunity and cell-mediated immunity. The ratio of $CD4^+/CD8^+$ can be taken as an important index to judge the state of organism's immune function [13, 14]. The ratio of $CD4^+/CD8^+$ in colon cancer patients decreases, which demonstrates that organism's immune function decreases. As immunosuppression cells, $CD4^+CD25^+$ regulatory T cells participate in tumors' immune escape and inhibit the proliferation and activation of $CD4^+$ and $CD8^+$ cells. NK cells are the essential component of organism's immunological surveillance system. Its number decides the strength of the immune function [15, 16]. In this research, after colon cancer patients' being treated by DC-CIK combined with chemotherapy, their $CD4^+$, $CD16^+CD56^+$ cell proportions and $CD4^+/CD8^+$ ratio are higher than those before treatment, and the proportions of $CD8^+$ T cells and $CD4^+CD25^+$ regulatory T cells are lower; besides, the improvement of all indexes are more significantly than that of chemotherapy group. All manifest that DC-CIK combined with chemotherapy can remarkably increase organism's immune function.

In addition, based on the secreted cytokines, T helper cells are classified to be Th1 and Th2 cells. They secrete different cytokines when functioning for coordination [17]. Th1 cytokines primarily enhance organism's cell-mediated immunity, including IL-2 and IFN-γ; Th2 cytokines largely functions for humoral immunity, including IL-6 and IL-10. IL-2 and IFN-γ secreted by Th1 cytokines can inhibit Th2 cell proliferation, while IL-6 and IL-10 secreted by Th2 cells can decrease the activity of Th1 cells, so immune system is inhibited, which creates microenvironment for the escape of tumor cells and metastasis [18-20]. Wang et al. [21] showed that DC-CIK treatment can increase the expression of IFN-γ in patients and decrease IL-10 level. In this research, the combined therapy leads to a significantly increased IL-2 and IFN-γ levels in patients and obviously decreased IL-6 and IL-10, which is consistent with previous results. It indicates that DC-CIK combined with chemotherapy can regulate Thl/Th2 balance in tumor patients. In conclusion, this study demonstrates that DC-CIK combined with chemotherapy can markedly improve immune functions of organism, increase survival rate, with a better clinical effect than chemotherapy alone.

Disclosure of conflict of interest

None.

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