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Assessment of serum tumor markers, tumor cell apoptosis and immune response in patients with advanced colon cancer after DC−CIK combined with intravenous chemotherapy

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ABSTRACT

Objective: To study the effect of DC-CIK combined with intravenous chemotherapy on serum tumor markers, tumor cell apoptosis and immune response in patients with advanced colon cancer. **Methods:** A total of 79 patients with advanced colon cancer conservatively treated in our hospital between May 2012 and October 2015 were retrospectively studied and divided into DC-CIK group and intravenous chemotherapy group according to different therapeutic regimens, DC-CIK group received DC-CIK combined with intravenous chemotherapy and intravenous chemotherapy group received conventional intravenous chemotherapy. After three cycles of chemotherapy, the content of tumor markers in serum, expression levels of apoptotic molecules in tumor lesions as well as immune function indexes were determined. **Results:** After 3 cycles of chemotherapy, CEA, CA199, CA242, HIF-1 α , IL-4, IL-5 and IL-10 content in serum of DC-CIK group were significantly lower than those of intravenous chemotherapy group; *p53, FAM96B, PTEN, PHLPP, ASPP2* and *RASSF10* mRNA content in tumor lesions of DC-CIK group were significantly higher than those of intravenous chemotherapy group; the fluorescence intensity of CD3, CD4 and CD56 on peripheral blood mononuclear cell surface of DC-CIK group were significantly higher than those of intravenous chemotherapy group while the fluorescence intensity of CD8 and CD25 were significantly lower than those of intravenous chemotherapy group; IL-2 and IFN- γ content in serum of DC-CIK group were significantly higher than those of intravenous chemotherapy group while IL-4, IL-5 and IL-10 content were significantly lower than those of intravenous chemotherapy group. **Conclusions:** DC-CIK combined with intravenous chemotherapy has better effect on killing colon cancer cells and inducing colon cancer cell apoptosis than conventional intravenous chemotherapy, and can also improve the body's anti-tumor immune response.

1. Introduction

Colon cancer is one of the common malignant tumors of digestive system in our country and its incidence is increasing year by year. Intravenous chemotherapy is the common adjuvant therapy for patients after colon cancer surgery and patients with advanced colon cancer, and chemotherapy drugs can kill cancer cells, delay the tumor progression and prolong survival time[1,2]. However, affected by adverse reactions and side effects of chemotherapy, the overall efficacy of intravenous chemotherapy is not satisfactory[3]. Immune escape is an important link that causes abnormal cancer cell proliferation and invasion in the body, patients with advanced colon cancer have the pathological state of reduced Numbers and weakened function of adaptive immune cells and innate immune cells, and chemotherapy drug damage to the immune response can aggravate the status of immune compromise, which increase the risk of immune escape of cancer cells[4]. Dendritic cell-cytokine-induced killer (DC-CIK) is the adoptive immunotherapy arisen in recent years, and it can enhance the body's immune function and kill tumor

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cells[5]. At present, there is no clear report about the effect of DC-CIK treatment of advanced colorectal cancer. In the following study, the effect of DC-CIK combined with intravenous chemotherapy for advanced colon cancer was analyzed from the aspects of serum tumor markers, tumor cell apoptosis and immune response.

2. Materials and methods

2.1. Research subjects

A total of 79 patients with advanced colon cancer treated in our hospital between May 2012 and October 2015 were selected, all patients were diagnosed with TNM IIIb-IV stage colon cancer by pathology biopsy, and patients combined with contraindications to chemotherapy and immunotherapy were excluded. The included patients were retrospectively analyzed and then divided into DC-CIK group and intravenous chemotherapy group according to the different therapeutic methods in medical records. DC-CIK group (n=32) included 21 male cases and 11 female cases, they were (53.5±6.8) years old, 23 cases were with TNM IIIb stage and 9 cases were with TNM IV stage; intravenous chemotherapy group (n=47) included 32 male cases and 15 female cases, they were (52.4±6.5) years old, 35 cases were with TNM IIIb stage and 12 cases were with TNM IV stage. The two groups of patients were not significantly different in general information.

2.2. Treatment methods

Both groups of patients accepted conventional intravenous chemotherapy, and the method was as follows: oxaliplatin, 130 mg/m^2, intravenous drip, within 2 h, on the 1st day, leucovorin calcium 200 mg/m^2, intravenous drip, within 4-6 h, on the 2nd-6th day, 5-fluorouracil, 500 mg/m^2, intravenous drip, within 4-6 h, on the 2nd-6th day, 21 days as one cycle of chemotherapy, for 4-6 cycles of chemotherapy according to patients' condition. DC-CIK group received DC-CIK treatment on the basis of conventional intravenous chemotherapy, and the method was as follows: peripheral blood was collected before intravenous chemotherapy to separate peripheral blood mononuclear cells and divide them into two, one was adjusted until the density reached 2 10^6/mL and then cultured for 2 h, suspension cells were removed, IL-4 and CM-CSF were added for processing, the medium was changed once every 2 days, IL-4 and CM-CSF were added when the medium was changed, and IFN-γ was added on the 6th day to promote DC maturation; the other was adjusted until the density reached 4 10^6/mL and then cultured for 2 h, suspended cells were obtained, cell density was adjusted again to 1 10^6/mL, IFN-γ was added for 24 h of culture, then IL-2 and CD3 antibodies were added, the medium was changed once every 2 days, and when the medium was changed, IFN-γ, IL-2 and CD3 antibodies were added to induce CIK maturation. DC and CIK were collected for 6 days of mixed culture at the proportion of 1:10, and the cells were collected, suspended with 2% albumin + 200 mL saline and transfused back to the body on the 14th-16th day of chemotherapy cycle, 1 time/day, for 3 consecutive days.

2.3. Detection methods of indexes in serum

After 3 cycles of treatment, 3 mL of peripheral venous blood was collected from two groups of patients and centrifuged to separate serum, then electrochemical luminescence kit was used to detect carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199) and CA242 content, and enzyme-linked immunosorbent assay kit was used to determine hypoxia-inducible factor-1 α (HIF-1 α), interleukin-2 (IL-2), interferon-γ (IFN-γ), IL-4, IL-5 and IL-10 content.

2.4. Detection methods of apoptotic molecules in tumor lesions

After 3 cycles of treatment, proper amount of tumor lesion tissue was collected by colonoscope, frozen by liquid nitrogen and then used for the extraction and detection of RNA, and the method was as follows: animal tissue RNA extraction kit was used to separate the RNA in colon cancer tissue, the first strand of cDNA synthesis kit was used to reverse-transcribe the RNA into cDNA, fluorescence quantitative PCR kit was used to amplify cDNA samples, the amplified genes included *p53, FAM96B, PTEN, PHLPP, ASPP2* and *RASSF10*, and the amplified gene mRNA levels were calculated according to the PCR curve.

2.5. Detection methods of immune molecules in peripheral blood

After 3 cycles of treatment, 3 mL of peripheral venous blood was collected and added in lymphocyte separation medium for density gradient centrifugation, middle suspension-layer mononuclear cells were extracted, peripheral blood mononuclear cells were re-suspended and adjusted until the density reached 1 10^7/L, fluorescent monoclonal antibodies of CD3, CD4, CD8, CD25 and CD56 were incubated respectively and then the fluorescence intensity of CD3, CD4, CD8, CD25 and CD56 were detected at flow cytometer.

2.6. Statistical methods

SPSS19.0 software was used to input and analyze data, measurement data analysis between two groups was performed by t test and P<0.05 indicated statistical significant differences.

3. Results

3.1. Tumor marker content of two groups of patients

CEA, CA199, CA242 and HIF-1 α content in serum of DC-CIK group were significantly lower than those of intravenous chemotherapy group (P<0.05) (Table 1).

3.2. Apoptotic molecule expression in lesions

p53, *FAM96B*, *PTEN*, *PHLPP*, *ASPP2* and *RASSF10* mRNA content in colon cancer lesion tissues of DC-CIK group were significantly higher than those of intravenous chemotherapy group (P<0.05) (Table 2).

3.3. Immune response of two groups of patients

The fluorescence intensity of CD3, CD4 and CD56 on peripheral blood mononuclear cell surface of DC-CIK group were significantly higher than those of intravenous chemotherapy group while the fluorescence intensity of CD8 and CD25 were significantly lower than those of intravenous chemotherapy group (P<0.05) (Table 3); IL-2 and IFN- γ content in serum of DC-CIK group were significantly higher than those of intravenous chemotherapy group while IL-4, IL-5 and IL-10 content were significantly lower than those of intravenous chemotherapy group (P<0.05) (Table 4).

4. Discussion

Adoptive immunotherapy is the malignant tumor treatment means arisen in recent years, and it mainly enhances the body's anti-tumor immune response to exert killing effect on cancer cells. DC-CIK is the most widely applied adoptive immunotherapy at present. DC is the most powerful antigen presenting cell in the body, there is reduced Numbers and weakened function of DC in most patients with malignant tumor, and exogenous amplification and supplementation of DC can enhance antigen presenting ability, promote T cell activation and exert anti-tumor effect; CIK has the characteristics of rapid proliferation, tumor lesion targeting and strong tumor cell killing, and exogenous amplification and supplementation o CIK can directly exert anti-tumor effect[6,7]. In order to define the effect of DC-CIK combined with intravenous chemotherapy for advanced colon cancer, the levels of serum tumor markers were w analyzed at first in the study. Tumor markers are a variety of molecules released and secreted by cancer tissues or para-carcinoma tissues in the occurrence and development malignant tumor, and they can reflect the tumor growth and load to a certain

Table 1
Comparison of tumor marker content in serum between two groups.

Groups	Case No.	CEA(ng/mL)	CA199(U/mL)	CA242(U/mL)	HIF-1 α (pg/mL)
DC-CIK group	32	11.78±1.94	46.53±7.14	29.54±4.15	126.58±15.48
Intravenous chemotherapy group	47	18.95±2.65	73.69±9.35	40.35±6.54	214.63±27.64
t		7.938	8.318	7.283	9.382
P		<0.05	<0.05	<0.05	<0.05

Table 2
Comparison of apoptotic molecule mRNA content in lesions between two groups (/ β *-actin*).

Groups	Case No.	*p53*	*FAM96B*	*PTEN*	*PHLPP*	*ASPP2*	*RASSF10*
DC-CIK group	32	2.28±0.33	1.93±0.24	2.09±0.28	3.28±0.41	1.94±0.22	2.76±0.33
Intravenous chemotherapy group	47	1.05±0.15	1.08±0.12	0.97±0.11	1.12±0.17	1.03±0.14	0.94±0.10
t		12.484	9.498	11.914	19.484	9.142	17.693
P		<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

Table 3
Comparison of fluorescence intensity of immune marker molecules on peripheral blood mononuclear cell surface between two groups.

Groups	Case No.	CD3	CD4	CD8	CD25	CD56
DC-CIK group	32	22.45±3.51	15.57±1.93	3.02±0.41	0.55±0.07	4.23±0.56
Intravenous chemotherapy group	47	12.49±1.65	7.38±0.94	4.23±0.65	1.04±0.13	1.87±0.23
t		8.948	10.498	6.685	9.449	16.541
P		<0.05	<0.05	<0.05	<0.05	<0.05

Table 4
Comparison of cytokine content in serum between two groups.

Groups	Case No.	Th1 cytokines		Th2 cytokines		
		IL-2 (pg/mL)	IFN- γ (pg/mL)	IL-4 (ng/mL)	IL-5 (ng/mL)	IL-10 (pg/mL)
DC-CIK group	32	28.76±3.52	0.93±0.11	93.52±10.32	147.24±21.35	1.42±0.18
Intravenous chemotherapy group	47	16.51±2.15	0.54±0.06	187.66±22.51	429.87±51.37	2.95±0.36
t		8.598	9.328	10.595	17.584	11.038
P		<0.05	<0.05	<0.05	<0.05	<0.05

extent. CEA, CA199 and CA242 are three tumor markers related to gastrointestinal malignant tumors, and are closely related to the progression of gastric cancer, colon cancer, pancreatic cancer and other malignant tumors[8]. HIF-1 α is a new tumor marker molecule discovered in recent years, and hypoxia is an important factor that induces increased HIF-1 α expression; Malignant tumor has higher metabolism rate and relatively hypoxic local special microenvironment, and hypoxia can increase the expression of HIF-1 α and then be combined with hypoxia responsible element through the HIF-1 α so as to increase the number of new blood vessels in malignant lesions and promote the growth of lesion group[9,10]. In the study, the comparison of the content of serum tumor markers between two groups of patients showed that CEA, CA199, CA242 and HIF-1 α content in serum of DC-CIK group were significantly lower than those of intravenous chemotherapy group. This means that the DC-CIK combined with intravenous chemotherapy has better killing effect on cancer cells than the conventional intravenous chemotherapy, and after treatment, the levels of serum tumor markers were lower and the tumor load was relatively smaller.

Promoting cell apoptosis is an important way for intravenous chemotherapy and immunotherapy to exert the killing effect on colon cancer cells, and the colon cancer cell apoptosis is regulated by a variety of molecules. p53, FAM96B, PTEN, PHLPP, ASPP2 and RASSF10 are the molecules that can promote colon cancer cell apoptosis. *p53, PTEN* and *PHLPP* are all tumor suppressor genes, and the encoded proteins can inhibit cell proliferation and induce cell apoptosis[11,12]; FAM95B is located in the spindle structure of cells, and can affect the stability of chromosome and cause cell apoptosis[13]; ASPP2 effect on promoting apoptosis mainly relies on p53, and ASPP2 can enhance the function of p53 and promote cell apoptosis[14]; RASSF10 affects cell cycle progression and inhibits cell proliferation mainly through Hedgehog and Wnt/ β -catenin pathways[15]. In the study, analysis of pro-apoptotic molecule expression in colon cancer lesions between two groups of patients showed that *p53, FAM96B, PTEN, PHLPP, ASPP2* and *RASSF10* mRNA content in colon cancer lesion tissues of DC-CIK group were significantly higher than those of intravenous chemotherapy group. This means that the DC-CIK combined with intravenous chemotherapy has better effect on promoting colon cancer cell apoptosis than conventional intravenous chemotherapy, and after treatment, pro-apoptotic molecule expression levels were higher in lesions.

Conventional intravenous chemotherapy exerts therapeutic effect mainly through the direct killing effects of chemotherapy drugs on cancer cells, but meantime, it will cause damage to normal tissue function, especially the immune function. The value of DC-CIK therapy is that DC cells have strong antigen presenting function and can enhance the body's own anti-tumor immune response, and that CIK has direct anti-tumor and cell killing properties and can directly exert anti-tumor immune response. T cells and NK cells are the main effector cells of anti-tumor immune responses, CD3, CD4, CD8 and CD25 are the marker molecules on T cell surface, CD3 can reflect the overall number and function of mature T cell, CD4 reflects helper T cell function, and the CD8 and CD25 represent the inhibitory T cell function; CD56 is the marker molecule on NK cell surface and can reflect the activation level and function of NK cells[16,17]. In the study, the analysis of the above immune marker molecule expression in peripheral blood showed that the fluorescence intensity of CD3, CD4 and CD56 on peripheral blood mononuclear cell surface of DC-CIK group were significantly higher than those of intravenous chemotherapy group while the fluorescence intensity of CD8 and CD25 were significantly lower than those of intravenous chemotherapy group. Th1 and Th2 subgroups in $CD4^+$T cells have the effect of mutual antagonism, and the shift the balance between the two to Th2 will cause immune suppression and tumor immune escape. Th1 cells mainly secrete IFN- γ , IL-2 and other cytokines, and Th2 cells mainly secrete IL-4, IL-5, IL-10 and other inhibitory cytokines[18]. In the study, analysis of serum levels of Th1 and Th2 cytokines between two groups of patients showed that IL-2 and IFN- γ content in serum of DC-CIK group were significantly higher than those of intravenous chemotherapy group while IL-4, IL-5 and IL-10 content were significantly lower than those of intravenous chemotherapy group. This means that the DC-CIK combined with intravenous chemotherapy can enhance the anti-tumor immune response in patients with colon cancer, helper T cell and NK cell function are significantly enhanced, and the inhibitory T cell function is significantly weakened.

To sum up, DC-CIK combined with intravenous chemotherapy has better effect on killing colon cancer cells and inducing colon cancer cell apoptosis than conventional intravenous chemotherapy, and can also improve the body's anti-tumor immune response.

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