

An experimental study on DC-CIK cells' immuno effects on killing gastric cancer cells *in vitro*.

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Abstract

Objective: To investigate the anti-gastric cancer immuno effects of DC-CIKs against gastric cancer cells by co-culturing Dendritic Cells (DCs) with Cytokine-Induced Killer cells (CIKs) derived from peripheral blood mononuclear cells of patients with gastric carcinoma.

Methods: Collecting 30 ml peripheral blood of patient of gastric cancer, we separate peripheral blood with lymphocyte separation liquid to obtain mononuclear cells respectively, and used for generating DC with the treatment of recombinant human Granulocyte Macrophage Colony Stimulating Factor (rhGM-CSF) and recombinant human Interleukin-4 (rhIL-4) as well producing CIK with interferon- γ (IFN- γ), CD3 and rhIL-2 *in vitro*, mixing SGC-7901 cells of DC and CIK cells and then DC and CIK were co-cultured according to the ratio of 1:10 to produce DC-CIK. The experiment was performed with three groups composed of DC-CIK group, DC group and CIK group. After 14 d of co-culture, increased number of cells were counted by trypan blue staining, cytotoxic activity against SGC-7901 cells in three groups were detected by MTT assay. The Flow Cytometry (FCM) was used to measure the antigen expression of the CD_{1a}, CD₈₃, CD₈₆, CD₈₀, CD₄₀, HLA-DR and the lymphocyte subsets of CD3+, CD3+CD8+ and CD3+CD56+ of the three groups cells. Levels of Interleukin-12 (IL-12), Interleukin-4 (IL-4), the Interferon- γ (IFN- γ) and Tumor Necrosis Factor- α (TNF- α) in the supernatants of DC-CIK, DC and CIK cells culture were detected by Enzyme-Linked Immunosorbent Assay (ELISA).

Results: After culturing for 14 d *in vitro*, the proliferation capability and the cells percentage of antigen expression of peripheral blood DC-CIK cells was significantly higher than that of DC and CIK cells ($p < 0.01$). The killing activity of DC-CIK cells was significantly higher than that of DC and CIK cells, with effect-target ratio increasing, the killing activity increased gradually ($p = 0.000$). CD3+CD8+, CD3+CD56+ double positive cells in DC-CIK co-culture were significantly increased than that DC and CIK groups under the same conditions ($p < 0.01$). The concentrations of IL-12, IL-4, IFN- γ and TNF- α in the cultural super mutant in the DC-CIK group were significantly higher than DC and CIK groups ($p = 0.000$). However, there is no statistics difference among the DC and CIK groups ($p > 0.05$).

Conclusion: DC-CIK can induce potent immunological effect against gastric cancer cells. Immuno efficacy of DC-CIK against gastric cancer is relevant to cytokine and cytotoxicity, which provides theoretical and experimental basis for the clinical immunotherapy of gastric cancer.

Keywords: Dendritic cells, Cytokine-induced killer cells, DC-CIK cells, Cytotoxicity, Cytokine, Gastric cells, Immunotherapy.

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Introduction

Gastric cancer is one of common malignant cancer in digest. The incidence of gastric cancer was rising in recent years in China. Surgery and chemotherapy were the effective treatment means of early gastric cancer. But there was no satisfactory treatment for those patients with advanced or metastatic gastric cancer currently. Many studies show that the immune function

in cancer patients was lower, and the tumor cells can evade the body's immune system to recognize through a variety of mechanisms [1]. Dendritic cells (dendritic cell, DC) was found the most functions professional antigen-presenting cells (antigen presenting cell, APC) in the body, which play an important role in the body's anti-tumor immune responses [2,3]. Cytokine-induced killer cells (cytokine-induced killer cells (CIK)) were acquired after co-cultured with human

peripheral blood mononuclear cells *in vitro* by a variety of cytokines (such as IFN- γ , IL-2, etc.) and CD3⁺ monoclonal antibody, which were kinds of heterogeneous cells composed by CD3⁺CD8⁺ and CD3⁺CD56⁺ double positive cells, which were one of the most optimal cells in current tumor immunotherapy in clinical application [4,5]. DC can recognize antigens and activate the acquired immune system. CIK can kill tumor cells through their cytotoxicity and cytokine secretion. But when the DC cells having a strong ability of tumor antigen-presenting were co-cultured with the CIK cells having a high efficiency killing activity, both can be adjusted to each other, CIK cells can promote DC maturation, while DC cells can enhance the proliferation and cytotoxic activity of CIK cells [6]. In this study, the PBMC (Peripheral Blood Mononuclear Cell) was isolated from peripheral blood in patients with gastric cancer, and then DC, CIK and DC were co-cultured to CIK (DC-CIK), which were induced and amplified cultured *in vitro*. We researched the phenotype, secreted cytokine and the cytotoxic activity to gastric cancer cells of DC, CIK and DC-CIK respectively to examine the immune effects to the gastric cancer cells of DC-CIK *in vitro* and provide experimental evidence and theoretical basis for the DC-CIK cells against gastric cancer treatment.

Materials and Methods

Reagents source

RPMI1640 cell culture medium and fetal bovine serum were purchased from Gibco Company; anti-Per-CPD3, anti-PE-CD56, anti-FITC-CD4, anti-APC-CD8, PE-CD86 mAb, FITC-CD 40 mAb were purchased from Becton Dickinson Company in United States, the determination of monoclonal antibody used in flow cytometry such as CD3, CD1a, CD83, CD80, CD86, CD40, HLA-DR, CD3CD4, CD3CD8, CD3CD56 and recombinant human interleukin-12 (rhIL-12), recombinant human leukocyte interleukin-4 (rhIL-4), recombinant human interferon- γ (rhIFN- γ), a recombinant human tumor necrosis factor- α (rhTNF- α), recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) were conducted by PeproTech Company. The ELISA kits purchased from PeproTech Company. MTT and lymphocyte separation medium (Ficoll) were purchased from Sigma company. The flow cytometry (FACS Calibur) were both purchased from BD Pharmingen Company in German. 311 TE incubator of CO₂ was purchased in TE companies in American, inverted microscope was produced by Olympus company in Japanese, human gastric cancer cell line SGC-7901 were supplied by the Central Laboratory in Third Military Medical University.

The subculture of human gastric cancer cells SGC-7901

The SGC-7901 cells were recovery cultured which were containing the medium with 10% RPMI 1640. Then the recovery SGC-7901 cells were subculture placed in 37°C with 5% CO₂ under saturated humidity conditions, after that, staining with trypan blue, counting the viable cell, and the

ration of living cells was required more than 95%. The cells in logarithmic growth phase were used for experiments.

Cell culture

Peripheral blood of 30 ml was collected from the patients with gastric cancer, obtaining PBMC through density gradient centrifugation by lymphocyte separation medium (Ficoll), then made it heavy suspension in RPMI 1640 medium with 10% fetal bovine serum and the cell concentration was adjusted to 6×10^6 /ml and then culturing in 37.0°C with 5% CO₂ in the incubator for 2 h.

(1) We obtained the adherent cells, which were cultured in complete medium with rhGM-CSF and rhIL-4 of 10 ng/ml. The medium was changed once a day in half-quantity, and at the same time adding cell growth factor and at the sixth day adding 50 U/ml of rhTNF- α in order to induce DC maturation, when the ration of CD11cCD83 double positive cell was more than 90%, the next test can conduct. Part of which was continue training by adding amplify culture solution, the DC cells were obtained after seven days' training, at this time we made cell phenotype analysis. Another part of DC cells were made induced culture adding rhIL-4 500 U/ml and rhGM-CSF 550 U/ml, at the same time added SGC-7901 cell lysate, which were co-cultured to the fourth day.

(2) Non-adherent cells were collected, the RPMI 1640 with 10% fetal calf serum was adjusting the cell concentration of 6×10^6 /ml, which were suspension culturing adding 1000 U/ml of IFN- γ , adding 1 μ g/ml CD3 monoclonal antibodies and 1000 U/ml of rhIL-2 after 24 h, medium was changed every two days in half-quantity, which were amplified cultured at a maintained cell concentration of 1×10^6 cells/ml by supplementing rhIL-2 and continued to train. Thereafter count every 2 d; furthermore the CIK was induced maturation by supplementing the culture solution adding rhTNF- α 50 U/ml at the sixth day. When the proportion of CD3CD56 double positive cells was more than 20%, the next test can conduct. Part of which were cultured for 7 d adding both amplification solution and SGC-7901 cell lysate. Other part of which were used to induction culture of CIK cells, collecting CIK cells after 14 d and then made phenotypic analysis.

(3) DC and CIK cells were respectively viable cell counted by trypan blue obtained at the seventh day of culture and adjust cell concentration to 1×10^6 /ml, which were co-cultured with CIK cell by a ratio of 1:10, the culture system is still the incubation system of CIK cell, the DC-CIK cells were obtained after 14 d of co-culture, which were used for the detection of biological characteristics for DC-CIK cells.

Grouping

The cells were grouped into three groups according the composition of effector cell: DC and CIK were co-cultured as the DC-CIK group, DC group and CIK group. The experimental conditions in three groups maintained consistency.

The detection of cell surface antigen

The cells were respectively prepared as suspension of 1×10^6 cells/ml in test tube of the flow cytometry, and then centrifugation with the speed of 500 r/min, abandon the supernatant and added PBS 100 μ l, wined and percussion for mixed, and then reserved cells. Three groups were both added the same type of negative control.

According to the instructions of labelled antibody in the BD company in US, the CD3-PerCP, CD4-FITC, CD8-PE and CD56-APC were added to the test tube and mixed, after that incubated darkly at 4°C for 30 min, the cells were washed three times with phosphate buffer (PBS), after that, they were suspended in 0.5 ml PBS and were added FITC-labelled CD3 antibody and PE-labelled CD8, CD56 antibody, which were incubated darkly at 4°C for 30 min, the expression of cell surface markers as CD3+, CD3+CD4+, CD3+CD8+ and CD3+CD56+ were detected by flow cytometry.

Analysis of activity of cell killing in vitro

The DC-CIK, DC, CIK cell having cultured for 14 d were selected as effector cells and the SGC-7901 cells in logarithmic growth phase were selected as target cells, the concentration was adjusted to 1×10^6 /mL, the ratio of effector cells to target cells was set as 2.5: 1, 5: 1, 10: 1, 20: 1, 40: 1, placed in 96-well plates. A separate group of target cells alone and effector cells alone was as a control group, each group set three wells and incubated for 8 h at 37°C with 5% CO₂, after that MTT of 10 μ l (5 g/l) was added to each well, continue incubated for 4 h, the centrifugal supernatant was removed, each well was added 100 μ l of dimethylsulfoxide (DMSO) to terminate the reaction, shocking for dissolved particles, detect the absorbance (A value) with microtiter detector measurement at a wavelength of 570 nm, the kill rate was calculated as follows: The killing rate=(1-(effector to target cell control efficiency in the experimental group A- A value)/target cell group A value) \times 100% .

The detection of cytokines

Culture supernatant 100 μ l having cultured for fourteen days in the groups of DC-CIK group, DC group and CIK group were seeded in 96-well culture plate respectively, which was removed after cultured in an incubator for 24 h, secretion of IL-12, TNF- α , IFN- γ and IL-4 in culture supernatants of each group were detected according with the operating requirements of IL-12, TNF- α , IFN- γ and IL-4 ELISA kit.

Statistical methods

Statistical software of SPSS 19.0 was applied, all the data are showed as ($\bar{x} \pm s$), the comparison of mean in two samples used paired t-test, and that in multiple samples used χ^2 analysis, test level $\alpha=0.05$, $P<0.05$ meant that the difference was statistically significant.

Results

Cell morphological observation

(1) The mononuclear cells obtained after cultured 3 h in the peripheral blood had the following texture that adherent cells were small and round; After 3 d cultured, the cells gradually became irregular in shape, tiny dendritic protrusions appeared in the surface; At the 5th d, volume became more larger, and the burr-like protrusions increased in the surface, some cells were initially suspended, which gathered into a cell groups of varying sizes and showed a typical of DC morphology.

(2) CIK cells cultured for three days were observed under inverted microscope that the number of cells increased and the volume some became larger, at the 6th d the speed of growth was found significantly faster and grew in in clusters and colony mode.

(3) DC and CIK were observed under a microscope after co-cultured.

That the proliferation of CIK cells were significantly faster, a large number of which were round, and the surround was shiny, the number increased significantly, while the number of irregular cells also significantly increased, suspension cells showed a significant cluster-like growth. cell count displayed by trypan blue viable that the number of DC-CIK cells were amplified to $96.38 \pm 6.51\%$, the number of DC cell were expanded to $68.86 \pm 6.57\%$, and that was expanded to $66.39 \pm 6.64\%$ on CIK cells, the number was respectively as thirteen times, six times, and eight times as the original one. The amplification times of cells compared in DC-CIK group and DC, CIK group, the difference was significant statistical significance ($p<0.01$).

The detection of cell surface antigen

The results detected by flow cytometry indicated that adherent cells in the peripheral blood of gastric cancer patients can be cultured mature DC, while non-adherent cells can be cultured mature CIK.

DC-CIK, DC and CIK cells were respectively cultured for 14 d, at this time the cell suspension were collected by flow cytometry, and the results showed that the expression of cell surface markers all showed high expression, and the rate of surface antigen markers on DC-CIK cell was significantly increased than that on DC and CIK cells ($p<0.01$, Table 1).

We found that the cells in three groups all showed high expression of CD3+ by detecting cell surface phenotype in three groups and the difference was significant statistical significance ($p<0.01$).

With the increasing of incubation time, the expression of double positive cells CD3+CD4+, CD3+CD8+ ratio for DC-CIK cells gradually increased.

Table 1. Expression rate of cell surface antigen in three groups ($\bar{x} \pm s$, %).

	CD _{1a}	CD ₈₃	CD ₈₆	CD ₈₀	CD ₄₀	HLA-DR
DC-CIK	72.45 ± 3.11	75.16 ± 2.24	74.52 ± 2.97	77.15 ± 2.77	76.32 ± 3.10	80.26 ± 3.45
DC	54.07 ± 2.41	52.32 ± 2.57	56.33 ± 2.82	58.06 ± 2.43	57.38 ± 2.47	60.49 ± 3.18
CIK	55.36 ± 2.54	49.22 ± 3.18	56.13 ± 2.84	55.04 ± 3.15	59.21 ± 2.77	61.42 ± 3.29
T value	-7.153	-8.204	-7.317	-8.036	-8.377	-8.251
P value	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 2. The cells percentage of CD3+, CD3+CD4+, CD3+CD8+, CD3+CD56+ in three groups ($\bar{x} \pm s$, %).

Group	CD3+	CD3+CD4+	CD3+CD8+	CD3+CD56+
DC-CIK	82.56 ± 4.94	28.45 ± 4.17	96.52 ± 7.33	85.39 ± 6.59
DC	75.35 ± 3.64	36.54 ± 3.69	78.41 ± 6.25	60.53 ± 6.13
CIK	77.47 ± 3.24	39.44 ± 3.55	75.57 ± 6.74	57.44 ± 6.37
T value	-8.254	29.583	-69.681	-62.512
P value	<0.01	<0.01	<0.01	<0.01

After the DC and CIK cells were co-cultured, the proportion of the double positive expression of CD3+ and CD3+CD4+, CD3+ CD8+, CD3+CD56+ on DC-CIK cells increased compared with DC and CIK cells, while the expression ratio of CD3+CD4+ cell subsets decreased compared with DC and CIK cells, the difference was significant statistical significance ($p < 0.01$, Table 2), and the difference between the group of DC and CIK group was not statistically significant ($p > 0.05$).

Table 3. Killing activity to gastric cancer cell SGC-7901 in different effector-target ratio in three groups.

Groups	Effector-target ratio				
	2.5:1	5:1	10:1	20:1	40:1
DC-CIK	20.35 ± 3.72	33.21 ± 3.54	45.19 ± 4.15	67.43 ± 5.17	89.52 ± 5.38
DC	15.32 ± 3.54	23.18 ± 3.61	36.22 ± 3.47	56.44 ± 7.30	77.43 ± 7.28
CIK	16.37 ± 4.16	25.29 ± 4.03	37.31 ± 3.52	58.45 ± 7.26	79.50 ± 6.74
T value	1.635	2.264	3.417	7.311	9.253
P value	0	0	0	0	0

Killing activity to gastric cancer cell SGC-7901 in different effector-target ratio in three groups

The cell killing activity of DC-CIK cells DC cells and CIK cells to SGC-7901 cell was measured by MTT assay, the results showed that: three effector cells all had high cytotoxic activity, and the killing activity was within the effective target range of 2.5: 1-40:1, which showed a positive correlation, just as that the greater ratio of effective target, the higher efficiency of killing activity. The killing activity of DC-CIK cell was strongest, which gradually increased with titers higher, the

difference was statistically significant compared with DC and CIK group, (p value=0.000, Table 3).

Detection of cytokines in three groups

Cell supernatants having cultured for fourteen days were collected of three groups, the levels of IL-12, IL-4, TNF- α and IFN- γ were detected with the ELISA kit. We found that concentration of IL-12, TNF- α IFN- γ secreted in three groups were higher and which were significantly increased in CIK group compared with DC and CIK group, the difference were statistically significant ($p=0.000$), especially in the secretion of IFN- γ was significantly increased ($p=0.000$). While the concentrations of IL-4 were lower cells secreted by the cells in the three groups compared with secretion concentration of IL-12, TNF- α and IFN- γ , the differences were statistically significant (p value=0.000, Table 4).

Table 4. The concentration of IL-12, IL-4, TNF- α and IFN- γ in the cell culture supernatant of three groups ($\bar{x} \pm s$, ng/l).

Group	IL-12	IL-4	TNF- α	IFN- γ
DC-CIK	107.25 ± 15.37	9.37 ± 8.53	457.46 ± 90.55	796.51 ± 183.74
DC	35.28 ± 17.49	10.42 ± 9.75	225.42 ± 78.19	349.61 ± 97.16
CIK	38.31 ± 17.53	11.51 ± 10.32	234.52 ± 80.23	375.64 ± 95.22
T value	4.473	2.356	7.187	12.295
P value	0	0	0	0

Discussions

DC was first discovered in 1973 by Steinman, which could uptake, process and handle antigens. It can effectively stimulate the initial and secondary immune response effector of T cells, and secrete IL-12 as the I cytokine of helper T cell type at the same time, which can also induce T cells and natural killer cells (natural killer, NK) to produce IFN- γ and enhancing the cytotoxic activity of NK activation by expressing type II histocompatibility antigens (major histocompatibility complex, MHC-II), co-stimulatory molecules, cell adhesion molecule-1 (intercellular cell adhesion molecule-1, ICAM-1) and lymphocyte function associated antigen-1, LFA-1) and other cell surface molecules *in vitro* and *in vivo*. which can effectively induce resting T cell proliferation, but also can promote cell cytotoxic T lymphocytes and helper T lymphocytes to generate and activate lymphocytes just as CD3+CD8+T and CD3+CD4+T to migrate to the tumor site,

after that it would secrete IL-12 and TNF- α , induce the production of IFN- γ by T cell and NK cells, further to develop anti-tumor effect [7]. DC can also affect the differentiation and proliferation of B cell; it can play a role in activation on the humoral immune response.

However, the DC function of patients with cancer was often defective, resulting in immune function lower or immune tolerance in patients, which is closely related to the progression, metastasis and recurrence of tumor [8]. CIK was a type of heterogeneous cells having CD3+CD8+ and CD3+CD56+ typically labelled, while expressing the surface marker CD3+CD56+T cell and NK cell, which was first reported in 1991 by Wolf et al. in Stanford University in USA [9]. CD3+CD56+T cells are the main effector cells of CIK population cells, which have both powerful anti-tumor activity of T cell and MHC restriction, kill the tumor characteristics of NK cells. It can also secrete high levels of IL-12, IFN- γ , TNF- α and other I cytokines of helper T cell type, to regulate the secretion body's other cytokines to achieve sensitivity of promoting CIK cytotoxicity on tumor cells, so as to restrain tumor and anti-tumor effects, which is an effective anti-tumor cells, and it can be induced to proliferate *in vitro* [10,11]. But it does not have the characteristics specifically recognize and kill tumor cells.

DC and CIK are two important cell for tumor immunotherapy, the former identified and presented antigens to induce the proliferation of CIK cell, at the same time which can promote the secretion of various cytokines to strengthen the anti-tumor effect; the latter can be directly or indirectly kill the tumor. To do this, the co-culture of DC and CIK can not only enhance the DC maturity, so as to play a stronger antigen commission ability, but also increases the cytotoxic effect of CIK cells, which will undoubtedly play a synergistic anti-tumor effect.

There are research reported that after co-cultured of DC and CIK cells from peripheral blood cell count of CIK was expansion fold at the fourteenth day, which was five times as that on the 7th d. But our experimental results showed that the number of DC-CIK cells on amplification and surface antigen expression were twenty times more than that for the number of DC and CIK cells after cultured 14 d, which indicated that DC can promote the proliferation of CIK cell in peripheral blood of human. The results are basically the same as Mortara et al. [12]. It affects cells of DC-CIK, DC and CIK cells in peripheral blood of gastric cancer patients though directly induce culture and co-culture for DC and CIK *in vitro* in the study.

The results of MTT test showed that the killing effect of DC-CIK was more strong than that of DC and CIK cells in same target cell with the same effect-target ration ($p=0.000$). While with the effector-target ratio (2.5:1~40:1) increasing, the killing effect of DC-CIK to target cell gradually enhanced ($p=0.000$). So we can speculate that when the concentration of cells increased in three groups, killing effect to gastric cancer cell would increase. Which show that DC-CIK induced to culture *in vitro* had a powerful killing effect to gastric cancer, the activity to anti gastric cancer was increased more compared

with DC and CIK cells, which was in line with the results about immune response of DC-CIK cells against nasopharyngeal carcinoma reported by Zhou in China, while it confirmed that DC-CIK cells have stronger activity against gastric cancer than DC and CIK cells. CIK was a type of heterogeneous cells and its anti-tumor effect was closely related with CD3+CD8+, CD3+CD56+ double positive cells and secretion of cytokines levels.

At the same time CIK cells had the advantage of antitumor activity of the T cells and the non-MHC restricted killing activity tumor of NK cells. The results of the detection to the DC-CIK, and DC and CIK cell subsets confirmed that DC-CIK generated more CD3+CD8+ and CD3+CD56+ than DC and CIK cell after culture *in vitro*. It means DC-CIK produce more cytotoxic lymphocytes.

The proportions of CD3+CD56+ in DC, CIK and DCIK cells were all more than 20%, but the proportions of the helper t-lymphocytes CD3+CD4+ helper a lower than that before inducing, which showed that the inducible factor added in induced-culture *in vitro* was benefit to the propagation of CD3+CD56+ and CD3+CD8+ and in enhanced cytotoxicity of CIK, as a result that improved its killing activity to gastric cancer cell. While the result also shows that the co-culture of autologous DC induced to culture *in vitro* can promote the proliferation of effector cells which has the CIK cytotoxic effector, and the increase the number of effector cells, so as to the CIK got a stronger activity killing activity to cancer.

DC and CIK cells were co-cultured, they both can respectively kill tumor cells by identifying the antigen, activating acquired immune system and playing their own cytotoxic activity and secreting cell factor, thus formed a highly efficient immune effect. On the one hand, it may be that CIK can kill tumor cells by exerting its own cytotoxicity, on the other hand, CIK and DC can identify and induce the secretion of IL-12 and IFN- γ each other, further to activate the cytotoxic activity of CD3+CD56+ cells and CD3+CD8+ cells to inhibit tumor growth [12].

Therefore, we detected the cell factor in the supernatant of the three groups of cells, and found that the concentration of IL-12, TNF- α and IFN- γ in the supernatant of DC-CIK group was significantly higher than that of DC and CIK, while the concentration of IL-4 was decreased ($p=0.000$). When DC is activated by immature state, it will secrete a large number of IL-12, IL-12, which not only mediates the immune response of T cells, promotes the proliferation and actives of cytotoxic T cells, but also induces IFN- cells and stimulates NK cells and helpful T cells to secrete IFN- γ , improve the cytotoxic activity of NK cells [13].

Thus, it can be inferred that the killing effect to gastric cancer cells in the co-culture of DC and CIK cells plays a synergistic effect, DC and CIK were identified each other, and the expression of cytokines and surface molecules was changed, DC promotes the proliferation of CIK cells and the extend the time of release and increase production of IFN- γ and TNF- α

and by secreting IL-12 [14]. Further to enhance the resistance of the DC-CIK activity of gastric cancer [15,16].

DC-CIK cells were showed having strong antitumor activity, which was directly or indirectly related to the number of CD3+CD56+ and CD3+CD8+ positive cells and the secretion of IL-12, IFN- γ , TNF- α and so on. IL-4 is a growth factor secreted by T cells, which can maintain the growth of helper T cells. As a kind of tumor immune modulator, the decrease of IL-4 secreted by DC-CIK may be one of the reasons for the non-proliferation of helpful T cells CD3+CD4+ in DC-CIK and IL-4 may not be involved in the anticancer effect of DC-CIK.

We speculated that secreting a large number of cytokines and producing cytotoxic cells may be one of the important mechanisms of DC-CIK cells against gastric cancer.

The research preliminarily affirmed that the effect of DC-CIK gastric cancer cells was obviously higher than DC and CIK cells, it can be used as an immunotherapy method of resistance to gastric cancer, what's more it provides experimental and theoretical basis for application of DC-CIK in biological immunotherapy of gastric cancer.

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