# Cytokine-Induced Killer Cells Co-cultured with Dendritic Cell Benefit for the Treatment of MCF-7/ADR--Mammary Cancer with Multi-Drug Resistance

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**ABSTRACT**

Cytokine-induced killer cells (CIK) are capable of lysing tumor cell and recognizing these cells in a non-MHC restricted fashion. After they were co-cultured with Dendritic cells (DC) loaded multidrug resistance tumor antigen, do they have the higher antitumor effect to the same multidrug resistance tumor?

**Methods:** The tumor-bearing mice model was established by MCF-7/ADR. DC and CIK were respectively cultured from peripheral blood mononuclear cells (PBMC) derived from healthy individuals. MCF-7/ADR was prepared to obtain the antigen lyses. CIK was co-cultured with DC pulsed or unpulsed by the tumor antigen lyses (AP-DC+CIK and DC+CIK). So there were AP-DC+CIK, DC+CIK and CIK groups, NS was used as control group. The tumor-bearing mice were injected by intravenous with the above 4 groups' effector cells. This study evaluated and compared the antitumor effect of different groups through the secretion of cytokines, the tumor size and the apoptosis of tumor cells in TUNEL test.

**Results:** After CIK co-cultured with DC, the frequency of CD3$^+$CD56$^+$ surface marker and the secretion of IFN-gamma, TNF-α and IL-2 were increased. After co-cultured, DC expressed the higher levels of mature markers, and secreted the more IL-12. The tumor size was the most inhibited in AP-DC+CIK group than in other 3 group. The TUNEL test had the significant differences between every two groups of 4 groups.

**Conclusion**: After CIK cells were co-cultured with DC loaded with multidrug resistance tumor antigen, the higher antitumor immune response was evaluated to multidrug resistance tumor expressed the same tumor antigen, which provide the effective clinical treatment to multidrug resistance tumor.

**Keywords:**   Cytokine-induced killer cells, dendritic cells, Multiple-drug resistance, MCF-7/ADR

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1

**INTRODUCTION**

Cytokine-induced killer cells (CIK) are non-major histocompatibility complex restricted cytotoxic lymphocytes generated by incubation of mononuclear cells, which derived from peripheral blood or bone marrow or cord blood, with anti-CD3 monoclonal antibody, interleukin (IL)-2, IL-1 and interferon gamma (IFN-γ). CIK represent cells with high antitumor cytotoxicity in vitro and in vivo, which involve secreting cytoplasmic granule and perforin, producing inflammatory cytokines (IFN-γ、TNF-α、IL-2, etc ), receptor ligand -mediated and so on. It has been shown that NKT cells co-expressing CD3 and CD56 markers on their surface represent the major cytotoxic subset of CIK cells (1). These NKT cells are derived from T cells (2). Dendritic cells (DC) are potent antigen presenting cell that recognize, process, and present antigens to T-cells in vivo and in vitro (3-5).They are key players in both innate and adaptive immune responses. DC-based immunotherapy has become one of the most promising approaches for the treatment of cancer (6, 7).The patients of malignancy relapsed after multiple chemotherapies or were resistant to chemotherapy, which possible reasons are the primary or secondary multidrug resistance (MDR) in the tumor cells. P- glycoprotein (P-gp) is the common reason of MDR, which was recognized as the tumor antigen (8, 9). In some human studies, the adoptive immunotherapy by effector cells was failed to some patients in malignancy, which must have been some connection with the absence of functional DC and the anergic in T cells. Some studies indicated that after DC co-cultured with CIK, DC was to be mature in morphology and function, CIK was increased in cytotoxicity and had high

proliferative response (10, 11). T cell also secreted more IFN-gamma under DC (12). Our former study (13) indicated CIK cells co-cultured with DCs pulsed by the tumor-lysate maybe could recognize the same tumor and have specific killing effect. So do CIK co-cultured with DC pulsed by multidrug resistance tumor antigen have higher antitumor cytotoxicity than CIK alone in nude mice? This study was to evaluate the antitumor immune responses of CIK associated with DC pulsed by multidrug resistance to the tumors expressed the same antigen and therefore showing an higher cytotoxic effect than CIK alone.

**METHODS**

**Target Cell**

MCF-7/ADR cells expressing about 90% P-gp antigen were purchased from institute of hemotology, Chinese academy of medical science.

**Tumor Bearing Mice Model**

MCF-7/ADR cells harvested in logarithmic growth phase were washed twice with normal saline (NS). They were centrifugated 1800rpm 5 mins, counted, resuspended in normal saline at $1\times10^7$/ml. SCID mice purchased in 6-8 weeks old were inoculated above cells 1ml per mouse at the right side of buttock. The tumor was formed when the cells became entity, which diameter was at least 0.5cm.

**Preparation of Freeze-Thawing Antigen**

MCF-7/ADR cells resuspended in above step were transferred into normal saline (NS), which concentration was 1ml cells into 100ul NS. They were put into liquid nitrogen

for 5 mins, then were transferred into 37°C bath immediately, then were put into liquid nitrogen again when they were melted completely, which were repeated 3 times. And the cells were collected by filtration, and kept in 4°C for standby.

**Generation of DC**

The peripheral blood mononuclear cells (PBMC) from healthy individuals were obtained by Ficoll-Paque (Sigma) density gradient centrifugation, and washed 3 times with PBS. These cells were cultured for two hour at 37°C in a humidified atmosphere of 5% $CO_2$ in RPMI-1640 (Hyclone Laboratories Inc) with 10% heat-inactivated fetal calf serum (Hyclone Laboratories Inc), 100 U/ml penicillin and 100μg/ml streptomycin. The non-adherent cells were collected for generating CIK cells. The adherent cells were allowed to adhere in twenty-four well plates at a density of $1 \times 10^6$ cells/ml in 1 ml RPMI-1640 with 10% heat-inactivated fetal calf serum, 1000 U GM-CSF(BD) and 500 U IL-4(BD), 100 U/ml penicillin and 100 μg/ml streptomycin per well for seven days for generating DC. The media along with the half of necessary cytokines were changed every two day. Part of DCs were pulsed with tumor-lysate antigen of MCF-7/ADR cells at 1:(3-5) ratio on day +5, which called AP-DC. All DCs were added TNF-α(BD) at 1000 U/ml for culture in one day on day +7. The morphology of DC was observed every 24 hours. DC mature markers were detected on day +8.

**Generation of CIK**

The non-adherent Ficoll separated human peripheral blood mononuclear cells derived from healthy individuals were prepared and grown in twenty-four well plates at a

density of $3 \times 10^6$ cells/ml in 1 ml RPMI-1640 medium with 10% heat-inactivated fetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin per well(14). One thousand U/ml IFN-γ (Jingmei Bioscience) was added on day 0. After 24 hrs of incubation, 50 ng/ml of anti-CD3 (Jingmei Bioscience), and 300 U/ml interleukin-2 (Jingmei Bioscience) were added. Cells were incubated at 37°C in a humidified atmosphere of 5% $CO_2$ and sub-cultured every third day in fresh complete medium with 300U/ml IL-2 per well. CIK cells were harvested on day +7 and were separated into 3 groups, 2 of them were co-cultured for seven days with AP-DC and DC at a stimulator (DC) to responder (CIK) ratio of 1:10, the other group was cultured alone for seven days. So the 3 groups were AP-DC+CIK, DC+CIK, and CIK groups. The markers of CIK cells were detected in 3 groups on day +15.

**Flow Cytometeric Analysis**

To confirm the maturation of the DCs, the harvested DCs were tested with CD1a-FITC, CD40-PE, CD80-FITC, CD83-FITC, CD86-PE, HLA-DR-APC (Becton Dickinson) before and after co-culturing in DC and AP-DC groups. The harvested CIKs were tested with CD3-FITC, CD56-PE, CD8-APC (Becton Dickinson) in AP-DC+CIK, DC+CIK, and CIK groups. Flow cytometry acquisition and analysis were performed with a FACScan using Cell Quest software (Becton Dickinson).

**Cytokines Detection**

The cell culture supernatants from the AP-DC+CIK and DC+CIK groups were sampled for IL-12 at the day +15 and the supernatant from AP-DC group was

sampled at the day +8, respectively. The cell culture supernatants from the AP-DC+CIK, DC+CIK, and CIK groups were sampled for IFN-γ, TNF-α and IL-2 at the day +15, respectively. The ELISA kit (Jingmei Bioscience) was performed according to the manufacturer's instructions.

**Injection Effector Cells to Mice**

After tumor formation in nude mice, the 40 mice were divided into 4 groups randomly, $1\times 10^8$ effector cells from AP-DC+CIK, DC+CIK, and CIK groups were injected into tumor-bearing nude mice through caudal vein, NS was to be control. The maximum transverse (a) and longitudinal (b) diameter of the tumor was measured per seven day ever since injection. The tumor size $=0.5\times a\times b^2$.

**Apoptosis Detection**

After the sections from the tumor were embedded with paraffin, they were dewaxed and dehydrated. The sections were incubated by protease K for 15-30mins at room temperature, and washed twice with PBS. The sections were covered with mixed solution of TUNEL(Roche) 50ul after drying, and incubated in the humidity incubator at 37℃ for 60 mins. Then the sections were washed 3 times with PBS. The sections were covered with POD transforming agents after drying again, and incubated in the humidity incubator at 37℃for 30 mins. Then the sections were washed 3 times with PBS. After the sections were covered with the DAB substrate solutions 50-100ul, they were incubated at room temperature for 10 mins, and washed with PBS again. The sections were counterstained, mounted, and analyzed by light microscope. Negative controls were set up at the same time.

## Statistical Analysis

Variance analysis was used for measurement data. $\chi^2$ test or rank test was used for enumeration data. P<0.05 means statistical significance.

## Results

**Table 1- CIK marker before and after co-cultured with DC**

| group | cell marker(%) | | |
|---|---|---|---|
| | CD3$^+$cells | CD3$^+$CD8$^+$cells | CD3$^+$CD56$^+$cells |
| CIK | 68.36±6.06 | 50.07±5.53 | 30.18±1.53 |
| DC+CIK | 79.62±5.23 | 69.43±4.04 | 49.13±3.50 |
| AP-DC+CIK | 96.37±8.38 | 83.83±3.06 | 66.07±2.63 |

DC and CIK cells were obtained after the peripheral blood mononuclear cells (PBMC) from healthy individuals were cultured through different methods. Then, CIK cells were separated into 3 groups after harvested on day +7 , 2 of them were co-cultured for seven days with AP-DC and DC at a stimulator (DC) to responder (CIK) ratio of 1:10, the other group was cultured alone for seven days. So the 3 groups were AP-DC+CIK, DC+CIK, and CIK groups. At the end of the culture, at least 1×104 cells were detected for the markers of CIK cells. The AP-DC+CIK group was the most, then was DC+CIK group, the last was CIK group. The statistical result was as follows: AP-DC+CIK:DC+CIK, P=0.003, SD=1.41; AP-DC+CIK:CIK, P=0.005, SD=4.07; DC+CIK:CIK, P=0.024, SD=4.56.

## Morphology and Marker on DC

**DC Mature Marker**

The costimulatory molecules, CD80 and CD86, and maturation marker CD83, all classic DC surface markers, were up-regulated during DC maturation (15). In the study DC mature marker expressed on AP-DC+CIK group was the highest, CD1a was 80.65±1.01%, CD80 was 89.62±2.23%, CD83 was 80.04±2.31%, CD86 was 91.35±1.17%, and HLA-DR was 92.05±6.20%.

**Table 2- DC Mature Marker Before and After Co-Cultured with CIK**

| group | CD40(%) | CD80(%) | HLA-DR(%) |
|---|---|---|---|
| DC | 29.22±5.04 | 30.35±7.36 | 31.47±9.21 |
| DC+CIK | 33.32±6.04 | 52.67±8.76 | 51.36±8.09 |
| AP-DC | 41.53±3.18 | 46.74±6.12 | 53.26±5.72 |
| AP-DC+CIK | 87.57±2.75 | 92.62±5.34 | 95.15±6.28 |

Morphologically, mature DCs are large cells with elongated and stellated processes(16). All DCs expressed mature morphology on day +15, and improved mature markers. AP-DC and DC co-cultured with CIK both expressed more mature markers than before, which differences had statistical significances (AP-DC+CIK:AP-DC, P=0.001,SD=2.35; DC+CIK:DC, P=0.012,SD=10.52).

**The Secretion of Cytokines**

The IL-12 secretion were performed in AP-DC, DC+CIK, and AP-DC+CIK groups, which results were 24.3±5.2pg/ml、41.2±9.5pg/ml、224±11.5pg/ml in turn. The IFN-γ secretion were performed in CIK, DC+CIK, and AP-DC+CIK groups, which results

were 436.4±8.9pg/ml、1237±76pg/ml、2897±212pg/ml in turn. The TNF-α secretion were performed in CIK, DC+CIK, and AP-DC+CIK groups, which results were 143.7±10.3pg/ml、509±83pg/ml、1049±133pg/ml in turn. The IL-2 secretion were performed in CIK, DC+CIK, and AP-DC+CIK groups, which results were 126.6±7.9pg/ml、232±39pg/ml、408±63pg/ml in turn.

**Table 3- Tumor Size in Tumor-Bearing Nude Mice Before and After Treatment**

| Group1 before | after | Group 2 | before | after | Group 3 | before | after | Group 4 | before | after |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 2.60 | 1.42 | 11 | 2.31 | 1.86 | 21 | 2.34 | 2.29 | 31 | 2.30 | 3.13 |
| 2 2.23 | 1.37 | 12 | 2.34 | 1.83 | 22 | 2.31 | 2.23 | 32 | 2.43 | 3.24 |
| 3 2.43 | 1.41 | 13 | 2.60 | 1.94 | 23 | 2.53 | 2.43 | 33 | 2.38 | 3.24 |
| 4 2.30 | 1.30 | 14 | 2.27 | 1.83 | 24 | 2.43 | 2.30 | 34 | 2.50 | 3.26 |
| 5 2.27 | 1.35 | 15 | 2.62 | 1.95 | 25 | 2.30 | 2.24 | 35 | 2.40 | 3.12 |
| 6 2.34 | 1.35 | 16 | 2.53 | 1.87 | 26 | 2.46 | 2.38 | 36 | 2.62 | 3.30 |
| 7 2.31 | 1.32 | 17 | 2.25 | 1.86 | 27 | 2.27 | 2.22 | 37 | 2.60 | 3.19 |
| 8 2.43 | 1.36 | 18 | 2.43 | 1.90 | 28 | 2.40 | 2.30 | 38 | 2.38 | 3.16 |
| 9 2.46 | 1.37 | 19 | 2.46 | 1.92 | 29 | 2.74 | 2.52 | 39 | 2.27 | 3.00 |
| 10 2.80 | 1.50 | 20 | 2.40 | 1.88 | 30 | 2.43 | 2.30 | 40 | 2.75 | 3.24 |

Group 1 was treated with the effector cells of AP-DC+CIK group(SD=5.681E-02).

Group 2 was treated with the effector cells of DC+CIK group(SD=4.248E-02). Group

3 was treated with the effector cells of CIK group(SD=9.562E-02). Group 4 was treated with NS(SD=2.788E-02). The DCs was not be removed from the effector cells' group mentioned above before each effector population was injected into tumor-bearing mice. The data had the significance differences between every two groups.

Tumor size was between $2.2cm^3$ to $2.8cm^3$ before treatment in tumor-bearing nude mice. Effector cells from treatment groups, which were AP-DC+CIK, DC+CIK, and CIK groups, were injected into tumor-bearing nude mice, NS was to be control. The tumors were still grown in mice treated by NS group, but the tumors treated by AP-DC+CIK group were inhibited obviously. The tumor sizes were between $1.3cm^3$ to$1.5cm^3$ in AP-DC+CIK group, but in NS group the tumor sizes were between $3cm^3$ to$3.3cm^3$ when executed mice after 1 month. The data had the significance differences between every two group(AP-DC+CIK: DC+CIK, P<0.01; AP-DC+CIK: CIK, P<0.01; AP-DC+CIK: NS, P<0.01; DC+CIK:CIK, P<0.01; DC+CIK:NS, P<0.01; CIK:NS, P<0.01).
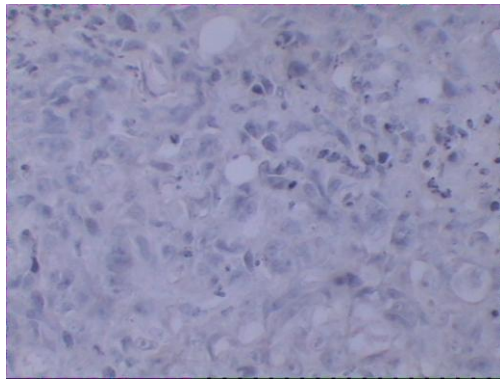
**Apoptosis Analysis by TUNEL**

Nucelus was stained in granular of deep yellow or deep brown colour under light microscope, which means apoptosis. According to the amount and distribution of apoptosis cells under light microscope (20×10), the apoptotic index (AI) was calculated with apoptosis cells divided by total cells in each high power field. According to the average of AI, which was calculated in 5 high power fields

randomly, negative( - ) means less than 0.1, weakly positive ( + ) means 0.1～0.2, moderately positive ( ++ ) means 0.2～0.3, strong positive ( +++ ) means more than 0.3. The difference between every two group had statistical significance (Table 4,figure 1).

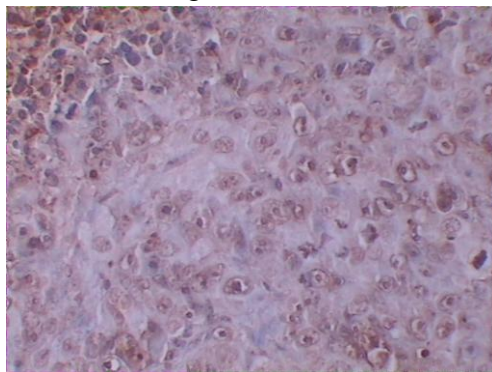**Table 4- Apoptotic Index Among 4 Groups**

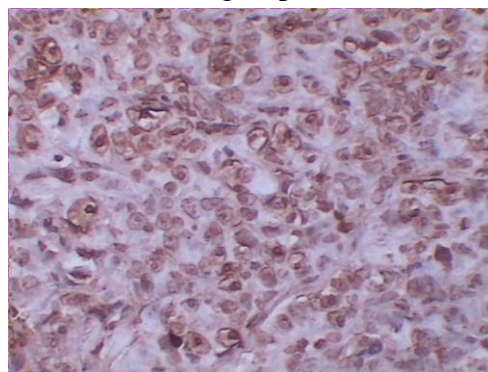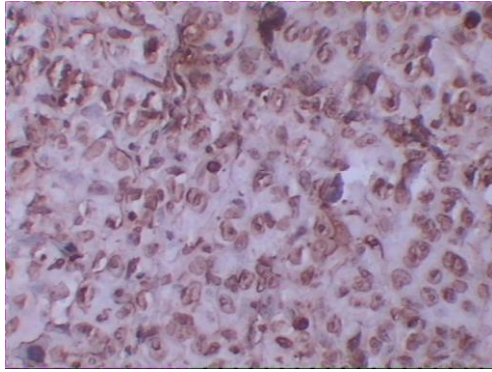| group | number | average AI |
|---|---|---|
| NS | 10 | 0.088±0.0103 |
| CIK | 10 | 0.29±0.0644 |
| DC+ CIK | 10 | 0.39±0.0409 |
| AP-DC + CIK | 10 | 0.562±0.0828 |



Negative Control
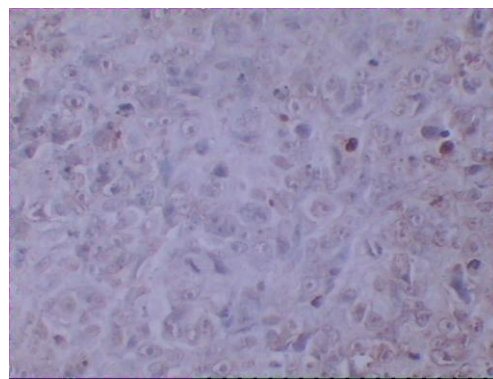


NS group



CIK group



DC+CIK group

AP-DC+CIK group

**Figure 1    Apoptotic Analysis Was Performed on TUNEL (×20)**

These pictures were the result of apoptotic analyse, which derived from the different treatment group of tumor-bearing mice. According to the different effector cells, the groups were AP-DC+CIK group, DC+CIK group, CIK group and NS group. These pictures were dealt with hematoxylin counter staining of TUNEL. Nucelus was stained in granular of deep yellow or deep brown colour under light microscope, which means apoptosis. According to the amount and distribution of apoptosis cells under light microscope, the apoptotic index (AI) was calculated with apoptosis cells divided by total cells in each high power field. The statistical result was that the difference between every two groups was significant (P<0.05).

**Discussion**

Chemotherapy is one of the most important methods for the treatment of malignancy. However, malignancy that initially respond to chemotherapy treatment develop

resistance over time. Notably, high levels of P-glycoprotein (P-gp) have been reported in patients experiencing multidrug resistance (MDR) (17, 18). Several mechanisms resulting in drugs resistance have been suggested. Among others the findings of ABC transport protein family suggests that P-gp may be of importance when resistance develops (19, 20).Thus, new approaches are necessary to improve the outcome of patients with malignancy suffering from MDR.

It is indicated that CIK have a higher antitumor toxicity as compared to standard lymphokine activated cells (2, 21, 22) and may be suitable to kill tumor cells resistant to chemotherapy(23). Therefore, they are promising candidates for the treatment of tumors with MDR. Dendritic cells (DC) play a key rule in recognizing, processing, and presenting antigens to T-cells in vivo (4,5). After interacting with DCs exhibiting the appropriate tumor-associated peptide antigen, CIKs are activated and expressed a functional status which could kill tumor cells that express these relative-molecules. We investigated whether CIK co-cultured with DC could benefit for the treatment of tumor expressed multi-drug resistance.

Regarding the proliferation of CIK cells, our study showed that CIK cells increased when compared to that before co-cultivation, specifically for AP-DC+CIK group which was almost 20-fold to CIK group. (data not provide).Co-expressing CD3 and CD56 markers, as well as CD3 and CD8 markers, on their surface represent the major cytotoxic subset of CIK cells (1). CD3CD56 double positive cells are thimbleful in peripheral blood lymphocytes（1%-5%）. In our study, there were significant difference of the frequencies of CD3CD56 double positive cells among these groups:

the highest one of that was in AP-DC+CIK group with a frequency of 66.07±2.63%, then the DC+CIK group with 49.13±3.50%, CIK group with 30.18±1.53% was lowest, AP-DC+CIK group was almost 2-fold to CIK group. The result was the similar with CD3CD8 double positive cells. All these results indicated that CIK cells strongly proliferated after co-cultured with DCs, and the proportions of CD3+CD56+ cells and CD3+CD8+ cells were increased accordingly, specifically when CIK cells interact with DCs loaded with peptide prepared by tumors expressed MDR (10). The cytokines ,which were IFN-γ, TNF-α and IL-2, were considered as the important cytokines of CIK, which improve the killing capacity of CIK cells to tumor cells. Marten A *et al.* detected that CIK cells co-cultured with DCs loaded antigen enhanced activation of proliferation and killing, increase the secretion of cytokine (IL-4, IFN-γ and so on)(10,24,25).Our study showed that the secretion of IFN-γ, TNF-α and IL-2 from CIK cells was stronger in AP-DC+CIK group than in DC+CIK and CIK group. Therefore, DCs loaded with specific peptide will benefit for the functional activity of CIK cells.

CIK cells have been shown to up-regulate DC specific markers which mark the mature of CDs(10). Molecular biomarkers that were associated with DCs maturation which could be used to characterize mDCs include HLA-DR, CD80, CD40. Our study showed that the expression of all 3 antigens increased during maturation, the expression of HLA-DR, CD80, CD40 was markedly increased in AP-DC+CIK group when compared to other two group. As an important cytokines related to DC functional, IL12 was tested to identified the function of mature DCs(26). The results

showed that the levels of IL12 in AP-DC+CIK group was almost 10-fold of that in AP-DC group, up to $224\pm11.5$pg/ml, indication that the maturation of DCs enhanced when co-cultured with CIK cells. The similar results was got by Marten *et al.* when DC loaded CA19-9(tumor associated antigen) co-cultured with CIK cells (10). DCs get activated when the ligand of CD40 of CIK is presented on the surface of the cells together with HLA and co-stimulatory signal, such as CD40、CD80, then secreting IL12, the supernatant from the cultivation of CIK cells can also promote the maturation of DCs.

Our former study (13) showed that CIK cells had different cytotoxic activities to the tumors with multi-drug resistance after cultured in 3 different groups in vitro. The first group was pulsed-DC+CIK that CIK cells co-cultured with DC who were pulsed with tumor-lysate antigen of MCF-7/ADR. The second group was DC+CIK, namely, CIK cells co-cultured with DC. The third group was CIK cells cultured alone. The above 3 groups were effector cells, while MCF-7/ADR and MCF-7 cells were target cells. The cytotoxic activities of the three CIK groups were detected by MTT after they killed target cells respectively. The cytotoxic activity to MCF-7/ADR had significant difference between any two groups, the highest is pulsed-DC+CIK group, and the lowest is CIK group. As far as MCF-7 was concerned, the CIK group had the lowest antitumor effect when compared with the other two groups, and the cytotoxic activities between the other two groups had no difference. These results indicated that CIK cells co-cultured with DCs pulsed by the tumor antigen with MDR maybe could recognize MDR tumor and have a specific killing effect. So we designed tumor model

study to further confirm the above conclusion.

Nude mouse were subjected to the modified MDR model. The effects of the CIK cells co-cultured with DCs on the treatment of tumors with MDR were evaluated by the sizes of tumor and cells apoptosis. The results showed that the application of the CIK cells co-cultured with DCs resulted in a decrease of 0.9-1.3 $cm^3$ averagely of the sizes in AP-DC+CIK group. However, as a control, the tumor in NS group grew fast. The data above indicated that CIK cells can kill the tumor cells and could be enhanced by the co-culture with DCs pulsed by the tumor antigen with MDR.

The apoptosis of tumor cells were measured by terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL). The AP-DC+CIK group shares a highest apoptotic index (AI), up to 0.562±0.0828, the other groups (DC+CIK, CIK and NS groups) were decreased respectively, the lowest one only 0.088±0.0103(Table 3 ,figure 3). There was a significance difference between every two groups. These results support the conclusion above that CIK cells co-cultured with DCs pulsed by the tumor antigen with MDR have a good outcome to the tumors with MDR.

One possible mechanism for CIK cells getting the capacity of killing the tumor cells after culture is CIK cells block cell cycle of tumor cells at S and G2/ M stage, inducing tumor cells apoptosis, depressing drug resistance gene expression, increasing the immunogenicity of resistant-drug cells, and secreting ant-ineoplasmic activity cytokine to kill tumor cells (27).Immunocytochemistry analysis of co-incubated CIK cells with RMS cells indicated that CIK cells do express TRAIL molecules which may ultimately lead to activation of caspase-3 in RMS cells, as a main caspase

responsible for the execution of apoptosis(28). It has been reported that up-regulation of activating CD94/NKG2C,CD94/NKG2D and CD94/NKG2E receptors in CIK cell population has a close relation with observed cytotoxicity (28,29). The mechanism for enhanced killing-effect of CIK cells to tumor cells with MDR after cocultured with DC is dependent on: ①DCs highly express MHC-Ⅱ molecules and costimulatory molecules, which can activate CIK cells by binding second signal. Thereafter, activated CIK cells enhance the affinity of LFA-1 to ICAM-1 through immunoglobulin Fc receptor, secrete endochylema toxic granulations containing BLT which initiate endochylema toxic granulation dependent dissolving cell effect(30); ② DCs secrete a lot of cytokines to activate the proliferation of antigenic specificity T cells, then, increase the activation and differentiation of CIK cells(31,32). After co-incubation with DCs loaded with tumor antigen with MDR, the increase in susceptibility of CIK cells to killing of the tumor cells with MDR was associated with the enhanced capacity of CIK cells to recognize specific antigen(33). Some study also proved CIK cells recognizes target cells through the T cell receptor (TCR) and requires the presence of MHC molecules on the target cells, which similar to that observed specific antitumor mechanism of classical cytotoxic T lymphocytes(34).

Together, this study suggests that CIK cells co-cultured with DC may be of clinical benefit in tumors with MDR. This technology will facilitate the development of adoptive immunotherapy of tumors with MDR.

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