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Original Article

The biological behavior of drug-resistantovarian cancer cells and changes in the CA125 and HE4 levels after CIK interventions

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Abstract: Objective: This study aimed to investigate the biological behavior of drug-resistant ovarian cancer cells and changes in the cancer antigen 125 (CA125) and human epididymal protein 4 (HE4) levels after the application of cytokine-induced killer (CIK) intervention. Methods: Drug-resistant ovarian cancer cells (namely SKVCR) were treated with CIK at different concentrations to observe the changes in the cell survival and cell morphology and the CA125, HE4, cytokine transforming growth factor-α (TGF-α), and tumor necrosis factor-α (TNF-α) levels in the cell lines before and after intervention. Results: With an increase in the CIK concentration, the survival rate of the SKVCR cell lines showed a decreasing trend. Under a constant CIK concentration, the survival rate of the SKVCR cell lines gradually decreased over time but become stable at 72 h. Before the CIK intervention, the SKVCR cells were full and rounded in shape, but after the CIK intervention, there was remarkable cell shrinkage and an increase in apoptotic cells. Compared with before the CIK intervention, the CA125 and HE4 levels were significantly decreased, but the TGF-α and TNF-α levels were increased ($P<0.05$). Conclusion: After the CIK intervention in the drug-resistant ovarian cancer cells, the cell survival rate decreases with an increase in the CIK concentration or an extension of the intervention time, and the cell morphology will be significantly improved, and the CA125, HE4, and other related cytokine levels will also change significantly, suggesting that CIK can kill drug-resistant ovarian cancer cells.

Keywords: Ovarian cancer, drug-resistant cells, CIK, biological behavior, CA125, HE4

Introduction

Ovarian cancer refers to malignant tumors that occur in the ovaries, about 90% of which are primary ovarian tumors, and the other 10% are metastatic tumors from other organs [1]. Ovarian cancer is the third most-common malignant gynecological tumor after cervical cancer and endometrial cancer. However, the mortality rate of ovarian cancer exceeds the sum of cervical cancer and endometrial cancer, ranking it first among gynecological tumors [2], and it is a major cancer threatening women's health. According to epidemiological data, there are 40 million new cases of ovarian cancer worldwide each year and 18 million deaths worldwide each year, which may due to the fact that the early clinical symptoms of ovarian cancer are subtle, so most patients miss the best time for treatment. The lesions are already in an advanced stage when diagnosed [3, 4].

Surgery, radiotherapy, and chemotherapy are common interventions for ovarian cancer, among which surgery is mainly used for patients with early-stage lesions but is ineffective in treating ovarian cancer in the advanced stages. Radiotherapy can inhibit the growth of tumor cells, but severe adverse reactions such as bone marrow suppression can significantly reduce patients' quality of life [5, 6]. Chemotherapy mainly kills tumor cells through drugs, which is relatively safe; however, clinical practice has shown that 80% of patients will achieve a significant clinical remission in the initial treatment, and there are still some patients who show no response to the initial treatment, or they suffer a relapse, which is a phenomenon of cell resistance [7, 8]. Cellular resistance significantly reduces the effectiveness of clinical treatment, prolongs the treatment cycle, and is detrimental to the improvement of patient prognosis. CIK cells are immunoreactive cells, first

reported by Schmidt in 1986, who experimented with the treatment of malignant tumors in animals in 1994, and CIK cells were applied to the treatment of leukemia in China for the first time in 1996. Clinical studies have confirmed that these cells have a better physiological effect at inhibiting tumor cell proliferation and accelerating tumor cell apoptosis [9-11]. The purpose of this study was to investigate the effects of CIK on the biological behavior of drug-resistant ovarian cancer cells and to analyze the effects of CIK on cellular CA125 and HE4 expressions in order to provide new strategies for the clinical treatment of multidrug-resistant ovarian cancer.

Materials and methods

Experimental materials

The SKVCR cell lines were purchased from Shanghai Meixuan Biotechnology Co. The CIK induction and culture were carried out in accordance with protocols established in previous studies. The DMEM/F12 medium, trypsin, and bovine serum were purchased from GIBCO, Inc. The study was approved by the Ethics Committee of the Affiliated Hospital of Jining Medical University.

Cell culture and intervention methods

The SKVCR cell lines were cultured in DMEM/F12 medium at 37°C, 5% CO_2, and the growth cells in their logarithmic phase were collected for the model establishment.

Outcome measurement

Measurement of the effect of the different concentrations of the CIK interventions on the viability of SKVCR cells using the MTT method: After the digestion and passage of the, well-growing SKVCR cells, the cells were inoculated into a culture plate at 37°C and 5% CO_2 for 12 hours. They were divided into a blank group and a study group. The blank group continued the routine culture, and the study group was treated with different concentrations of CIK (0.1 mM, 0.5 mM, 1.0 mM, and 2.0 mM), and the cell viability was measured after 12 hours of incubation.

Measurement of the effects of the different interventions on the viability of the SKVCR cells using the MTT method: After 12 h of culture at 37°C and 5% CO_2, the cells were divided into a blank group and a study group. The blank group was cultured routinely, and the study group received 1.0 mM CIK for intervention, and the cell viability was measured using enzyme labeling at 0 h, 6 h, 12 h, 24 h, 48 h and 72 h, respectively.

Observation of cell morphology changes before and after intervention: After 12 h of culture at 37°C and 5% CO_2, the cells were divided into a blank group and a study group. The blank group was cultured routinely, while the study group was treated with 1.0 mM CIK, and the morphology of the two groups was examined using electron microscopy after 12 h as follows. The cells were scraped off , centrifuged at 3000 r/min for 5 min, rinsed once using a phosphate buffer, and then placed in a centrifuge tube for a 0.5-hour fixation using glutaraldehyde. The fixed cell pellets were scraped off with acetone for dehydration, and epoxy resin for embedding. The cell morphology was observed after the cells were stained with uranyl acetate and lead citrate.

Changes in the cytokine levels before and after the intervention: The cytokine CA125, HE4, TGF-α, and TNF-α levels were observed before and after the intervention with 1.0 mM CIK for 12 hours. The CA125 and HE4 levels in the cell suspensions were measured using electrochemiluminescence, and the TGF-α and TNF-α levels were measured using enzyme-linked immunosorbent assays (ELISA). All the kits were purchased from Quanzhou Ruixin Biotechnology Co., Ltd. The measurements were carried out in strict accordance with each kit's instructions. Each sample was tested 3 times, and the average value was taken as the final result.

Statistical methods

The collected data were entered into an EXCEL sheet. SPSS 20.0 software was used to analyze the data, and normal distribution tests were carried out on the collected data. If the data conformed to a normal distribution, the count data were expressed as [n (%)]. Chi-square tests were used to analyze the group differences, and the measurement data were expressed as the mean ± standard deviation (mean ± SD). t-tests were used for the comparisons, and t-tests were used for the analyses of the continuous variables. Student's t tests were used for the comparison of the differences,

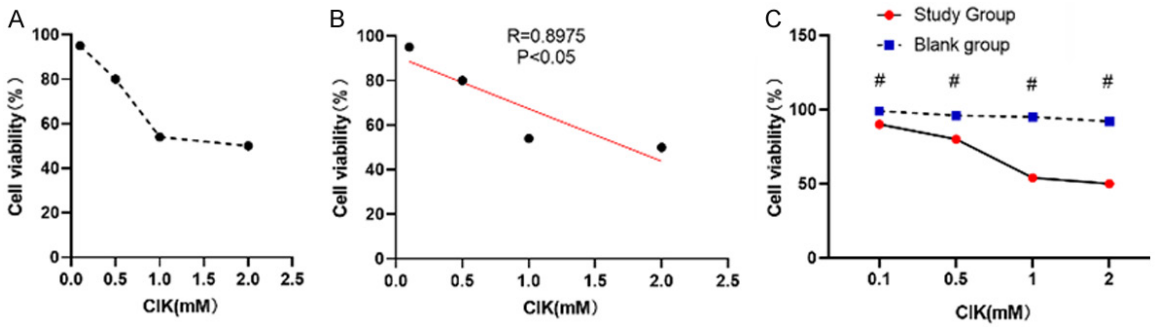

Figure 1. The effect of the different concentrations of CIK intervention on SKVCR cell survival. As the concentration of the CIK intervention increased, the survival rate of the SKVCR cells showed a clear decreasing trend, and the higher the concentration of CIK, the lower the SKVCR survival (A). The SKVCR cell survival rate showed a significant negative correlation with the CIK concentration (r=-0.8975, $P<0.05$) (B). The cell survival rate in the study group was lower than it was in the blank group ($P<0.05$) (C). # indicates that the difference of the same indicator between the groups is statistically significant.

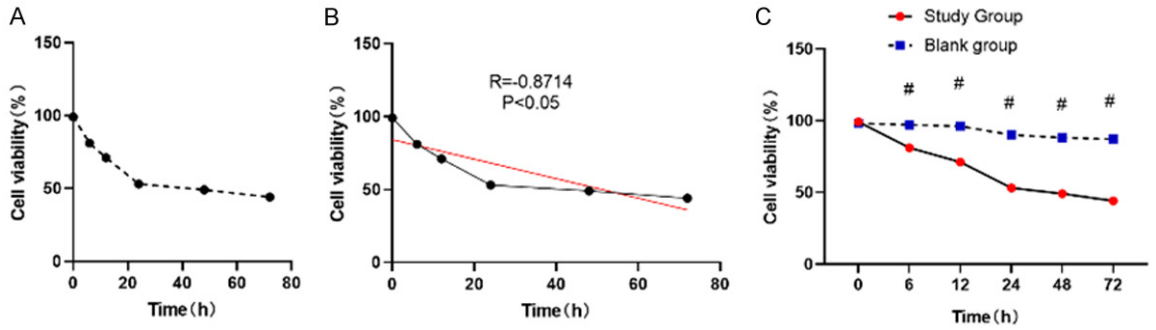

Figure 2. The effects of the different intervention times on the survival rates of the SKVCR cells. The effects of the CIK intervention times on the SKVCR survival rate (A); A Spearman correlation analysis found that the survival rate of SKVCR cells was significantly negatively correlated with the CIK intervention time (r=-0.8714, $P<0.05$) (B). Comparisons between two groups (C); # indicates that the difference in the same indicator between groups is statistically significant.

and Spearman's analysis was used for the correlation analyses. $P<0.05$ was considered a significant difference [12].

Results

The effect of the drug concentrations on the viability of the SKVCR cells

With an increase in the concentration of the CIK, the survival rate of the SKVCR cells showed a remarkable tendency to decrease. The higher CIK concentration the lower the survival rate of SKVCR. Our Spearman correlation analysis revealed that the survival rate of the SKVCR cells showed a significant negative correlation with the concentration of CIK (r=-0.8975, $P<0.05$), and the survival rate in the study group under CIK intervention at each concentration was significantly lower than it was in the blank group (**Figure 1**).

The effect of the different intervention times on the SKVCR cell viability

The Spearman correlation analysis showed that the SKVCR cell survival rate showed a significant negative correlation with the duration of the CIK intervention (r=-0.8714, $P<0.05$). The survival rate of the cells in the study group was lower than the survival rate of the cells in the blank group at 6 h, 12 h, 24 h, 48 h, and 72 h under 1.0 mM of CIK intervention (**Figure 2**).

Observation of cell morphology changes before and after the intervention

The two groups of SKVCR cells were observed using electron microscopy, and there were no

Table 1. Changes in the cellular tumor factor levels before and after the intervention ($\bar{x} \pm s$)

Group	CA125		HE4	
	Pre-intervention	Post-intervention	Pre-intervention	Post-intervention
Study group	289.89 ± 30.19	160.29 ± 19.29	167.76 ± 20.98	110.76 ± 9.87
Blank group	290.88 ± 29.18	200.28 ± 14.87	170.33 ± 18.56	134.78 ± 10.28
t	0.149	10.384	0.58	10.66
P	0.882	<0.001	0.564	<0.001

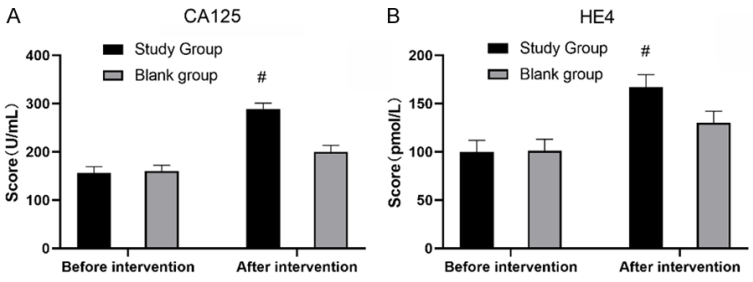

Figure 3. Changes in the cellular tumor factor levels before and after the intervention. CA125 (A) and HE4 (B). # indicates compared with the blank group, the difference in the same indicator is statistically significant.

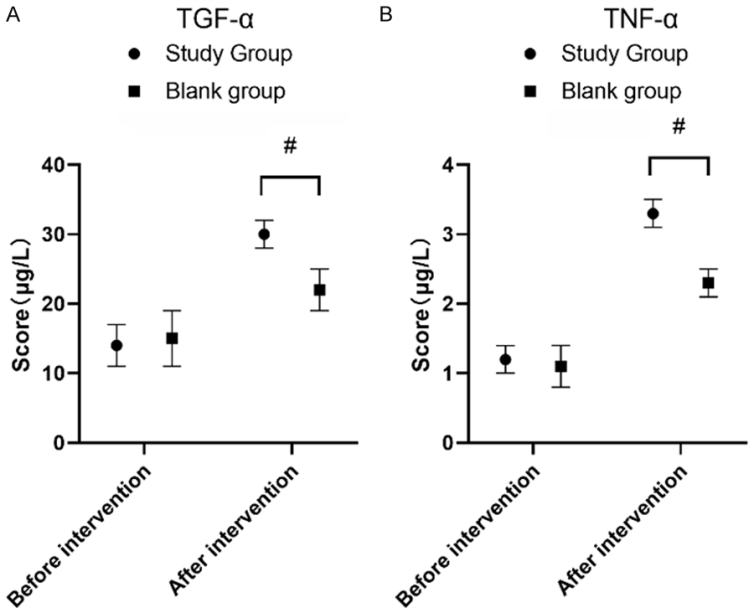

Figure 4. Changes in the cellular inflammatory factor levels in the two groups before and after the intervention. TGF-α (A) and TNF-α (B). # indicates that the difference between groups at the same time is statistically significant, *P*<0.05.

Changes in the tumor necrosis factor levels before and after the intervention

There was a significant difference in the CA125 and HE4 levels in the SKVCR cells in the two groups before the intervention (P>0.05). After the intervention, the CA125 and HE4 levels in the study group's cell lines showed a significant decrease, and they were lower than their pre-intervention levels (*P*<0.05). The tumor factor levels of the cells in the study group were lower than they were in the control group (*P*< 0.05) (**Table 1**; **Figure 3**).

Changes in the cellular inflammatory factor levels before and after the intervention

There was little difference in the TGF-α and TNF-α levels in the SKVCR cells in the two groups before the intervention (*P*>0.05). After the intervention, the TGF-α and TNF-α levels in the study group's cell lines showed a significant increase (*P*<0.05), while the comparison between the groups showed higher cellular inflammatory factors levels in the study group (*P*<0.05) (**Figure 4**).

Discussion

significant differences in the cell morphology before the CIK intervention. After the CIK intervention, the cells showed a significant decrease in volume, the proportion of apoptotic cells was high, and the mitochondrial concentrated chromatin was shrunken around the nuclear membrane, presenting half-moon morphology.

About 60%-70% of patients with ovarian cancer are in an advanced stage when diagnosed. The 5-year survival rate of ovarian cancer patients is only 30%. The incidence rate has been increasing due to unhealthy lifestyles and the aggravation of environmental pollution. Traditional treatments for ovarian cancer include

surgery, radiotherapy, and chemotherapy. Both surgery and radiotherapy are limited in efficacy [13, 14]. Chemotherapy is mainly based on cytoreductive surgery and platinum drugs, and although platinum drug intervention has a defined effect, some patients still experience recurrence or a poor prognosis, which may be related to the development of drug resistance in ovarian cancer cells. Therefore, new interventions to reverse chemotherapy resistance have become a crucial direction in ovarian cancer treatment [15, 16].

Clinical practice has shown that DNA damage repair pathway is involved in drug resistance in ovarian cancer cells, and platinum drugs mainly affect the proliferation and division of ovarian cancer cells through nucleotide excision. However, excision repair cross-complementing groups can repair the DNA damage and promote the proliferation and division of ovarian cancer cells, so intervention targeting this process is an important means to reverse drug resistance in ovarian cancer cells [16, 17]. In recent years, with the development of molecular biology and tumor immunology, cellular immunotherapy has been applied as an adjuvant therapy, which has improved the clinical efficacy of cancer therapy [18]. CIK cells, also known as natural killer cell-like T cells, are a kind of composite cell that combines the killing activity of T cells with the efficacy of natural killer cells. CIK cells can kill a variety of tumor cells and have little influence on the autoimmune process. Some scholars conducted studies on the intervention effect of CIK on different tumor cells, and the results showed that CIK has a good growth inhibition effect on different malignant cells such as breast cancer, gastric cancer, and liver cancer, and the growth-inhibition rate in the breast cancer cells was as high as 51.7% [19]. Another study pointed out that the incidence of GVHD in patients during the process of CIK cell reinfusion was only 11%, and there were no adverse reactions of grade III or above, suggesting that the CIK intervention is safe [20]. Studies has found that CIK has strong oncolytic activity, but there is no MHC limitation in T lymphocyte killing, and in vitro experiments show that it exhibits a strong killing power in a variety of tumor tissues, so it is significantly more effective than LAK cells [21, 22].

The results of this study showed that the survival rate of the drug-resistant ovarian cancer cells with different CIK concentrations was significantly lower than of the rate in the blank group, and with an increase in CIK concentration, the survival rate showed a gradual downward trend. A correlation analysis found that the CIK concentration was negatively correlated with the survival rate of the drug-resistant ovarian cancer cells. Some scholars have conducted research on the influence of CIK on the biological characteristics of malignant cells through in vitro experiments, and the results showed that different CIK intervention concentrations had different killing effects on the malignant cells, but the killing effect would not always increase with an increase in the concentration. The killing effect is the best at a concentration of 1.5 mM, a finding similar to the results of this study [23]. The reason may be that CIK has a broad anti-tumor spectrum, a rapid proliferation rate, and high tumor killing activity. CIK can not only directly release perforin, granzyme, and other substances to induce lysis of tumor cells, but it also plays an indirect killing role by regulating cellular immune function, and significantly reducing the cell survival rate under various mechanisms [24]. We found that the longer the intervention time, the lower the survival rate of the drug-resistant ovarian cancer cells. This has also been confirmed in various studies. However, there is a significant difference in the survival rates of the drug-resistant ovarian cancer cells between 0-20 h and 40-60 h. The reason may be that the drug-resistant ovarian cancer cells show a certain degree of adaptability, or the CIK at a concentration of 1.0 mM does not exert its maximum killing effect.

We also found that the CA125 and HE4 levels in the cell lines showed a significant decrease, and the levels of TGF-α and TNF-α levels showed a significant increase after the CIK intervention, suggesting that the CIK intervention would regulate the tumor cytokine and inflammatory factor levels in the organism. A study conducted on elderly patients with multiple myeloma receiving chemotherapy showed that the immune function and inflammatory factor levels were significantly altered after the CIK intervention, with significant reductions in the CD3+ and CD8+ levels as well as the IL-6 and TGF-β levels, which differed from the results in this study [25]. It is speculated that the difference may be related to the mechanism CIK uses to kill the tumors. CIK can

enhance the cytotoxicity by increasing the inflammatory factor levels, which then has a killing effect [26]. The effects of CIK on the inflammatory factor levels remains to be further explored.

In summary, the survival rate of drug-resistant ovarian cancer cells will decrease with an increase in the CIK concentration or intervention time, and the cell morphology will be significantly improved, while the CA125, HE4, and other related cytokine levels will also change significantly, suggesting that CIK is effective at killing drug-resistant ovarian cancer, so it can be considered for clinical treatment. The innovation of this study lies in its analysis of the effects of the different concentrations and different intervention times on cell viability. At the same time, the effect of CIK on the biological behavior of drug-resistant ovarian cancer cells was demonstrated in detail through an observation of cell morphology using electron microscopy and an analysis of the cell tumor factor levels. The data are detailed and reliable, so they can provide a theoretical reference for follow-up intervention. The shortcomings of this study are that, although the effect of CIK on the biological behavior of drug-resistant ovarian cancer cells has been analyzed, the underlying mechanism was not fully explored, so there is a lack of a molecular level analysis. The next step is to carry out more in-depth molecular studies.

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Disclosure of conflict of interest

None.

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