

# Feasibility of Using Adoptive Cell Immunotherapy to Target Differentiation of CD4+T Cells and Co-culture of DC-CIK to Kill Tumor in Vitro

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## Abstract:

Tumor immunotherapy is a hot topic in the field of tumor therapy today. The use of CD4+T cells to enhance the killing effect of CD8+T cells and the use of DC cells for local immunotherapy are excellent methods of tumor immunotherapy, but no matter which method still have its own defects, CD4+T cells have a dual effect on CD8+T cells, and will differentiate into both tumor promoting and tumor-inhibiting phenotypes in the human body. Due to the scarce number of DC cells, it is difficult to achieve effective inhibition effect. This article mainly discusses the feasibility of in vitro culturing through adoptive cellular immunotherapy (ACI) to extract CD4 + T cells, DC and CIK cells that are inside body to solve the above problem. At the same time, the differentiation of CD4+T cells can be stronger because in vitro culture can carry out directional catalysis on single cells and avoid the influence on non-target cytokines. DC and CIK cells can play the role of local tumor inhibition and tumor killing respectively, and the combined culture can enhance their anti-tumor effect, but the number in vivo is very small, resulting in no obvious effect, the two in vitro multiplication and parallel culture can produce a stronger anti-tumor effect. Therefore, the safety and efficacy of this method in clinical application as well as the prognosis of patients still need to be further observed, and further studies can be conducted in the aspects of postoperative adaptation of ACI clinical patients.

**Keywords:** adoptive cell immunotherapy, CD4+T cells, DC-CIK1,Co-culture, Th1/Th2

## 1. Introduction

Cancer is currently one of the most difficult human diseases to cure. Cancer occurs when cells overexpand due to abnormal mechanism of apoptosis. The unrestricted growth of cells without following their normal mechanism of apoptosis is a great burden to the human body, and such abnormal apoptosis or death cells are cancer cells, which are cells with abnormal apoptosis mechanism. Therefore, in essence, the occurrence of cancer is a disease that causes great loss to the human body due to the abnormal immune system leading to the non-death of cells. Adoptive cell immunotherapy (ACI) can extract cells that have a killing effect on the tumor in vitro for targeted catalysis in the beneficial direction of tumor treatment and then transport them back to the body, so as to enhance the patients' own immune system in order to effectively control the tumor. This is a method of taking one's own cells, cultivating them outside the body and then re-injecting them back

into the body to produce an effect. With this method, the effect of inducible factors on non-intended cytokines can be avoided. CD4+T cells have double effects on CD8+T cells, and different subtypes have different effects. Th1 promote CD8+T cells and Th2 inhibit CD8+T cells. The importance of studying the mechanism of Th1/Th2 differentiation lies in its ability to enhance the killing of CD8+T cells on tumors. Current researchers have recognized the importance of this [1], but a perfect Th1/Th2 catalytic balance mechanism has not been explored yet. Due to the small number of DC cells and CIK cells, it is difficult to independently produce a large area of tumor inhibition effect, but the co-culture of DC and CIK cells in vitro can promote the maturation of DC and enhance the tumoricidal activity of CIK cells (increasing the number of effective tumoricidal cells in expanded CIK cells in vitro) [2]. Therefore, the use of adoptive immune cell therapy to outgrow DC and CIK cells and then inject them back into vivo can highly enhance their destruction of tumor

cells. The clinical safety and efficacy of this therapy, the adaptability of different patients to this therapy, and the therapeutic prospect of this therapy still need attention and exploration. Therefore, this article reviews the existing treatment methods of ACI and the application of CD4+T cells to CD8+T cells, DC, CIK cells and ACI binding immune cells were studied, aiming to find the advantages of ACI on human anti-cancer and clinical practice effects.

## 2. The Differentiation of CD4+T Cell Subtypes and the Effect and Clinical Application of DC on Tumors

### 2.1 The Role of CD4+T Cells on Tumors

The immune system is an important system for the body to fight diseases and has many cells including CD4+T cells. CD4+T cells are a type of helper T lymphocyte that has a dual role for CD8+T cells as killer cells, and there are many subpopulations, including Th1, Th2, Th9, Th17 and others. Different subtypes of lymphocytes secrete different cytokines and produce different types of immune responses. As shown in Figure 1, the Th1 subtype promotes tumor growth, while the Th2 subtype inhibits tumor growth. When the Th2 subtype increases, its cytokines inhibit the differentiation of helper T lymphocyte subsets to Th1 subtype [3]. Th1 and Th2 are the two main types of Th cells. Under normal circumstances, both of them can multiply and develop to form a balance, but when there is a tumor growing in the body, the imbalance between them will be caused, and the number of Th2 will increase and inhibit the differentiation of Th1 [4]. CD4+T cells in peripheral blood can be isolated by the research method of Zhao Qian et al. [2], through EDTA anticoagulation, plasma removal by centrifugation, blood cells removed from plasma added to lymphocyte separation solution and centrifugation, peripheral blood mononuclear cells can be obtained. It can be found that Th1/Th2 imbalance is the main mechanism of the development of most tumors. According to the research of Zheng Yingchun et al. [5], it can be seen that when the tumor is inhibited, the amount of IFN- $\gamma$  and other cytokines secreted by Th1 type increase, which represents the increase of Th1 cells, which proves that the catalytic transfer of CD4+T cells to Th1 type has an effect on tumor inhibition. Through adaptive cell transfer immunotherapy, immune cells CD4+T cells are extracted from the patient and activated in vitro for targeted subtype differentiation and then transfused back into the patient to enhance the patients' autoimmunity and achieve the effect of tumor cell killing, which is promising to cure the tumor. However, it is still necessary to pay more attention to the prognostic adverse reactions and find ways to solve the adverse reactions.

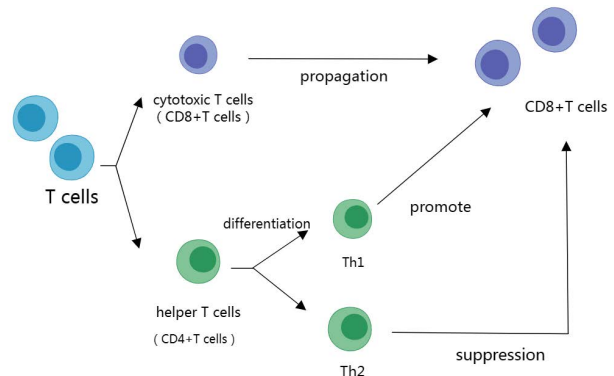


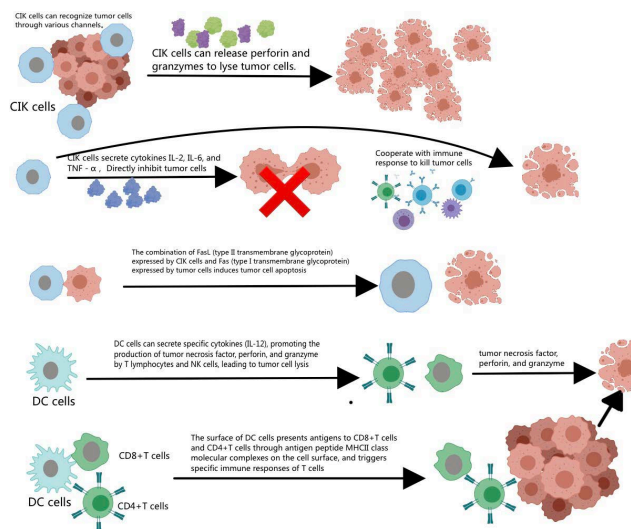
Fig. 1 Effect of CD4+T cell differentiation on CD8+T cells

### 2.2 The Role of DC Cells on Tumors

DC cells and CIK cells are two important immune cells, but the number of DC in vivo is rare and mature cells are few. When CIK cells are amplified alone in vitro, only some cells such as CD3+, CD8+ and CD56+ have anticancer effects, and these cells only account for a small number of amplified cells [6]. As shown in Figure 2, co-culture of DC and CIK cells in vitro can promote the maturation of DC and enhance the tumor-killing activity of CIK cells. CIK cells are a heterogeneous cell population with high expression induced by a variety of cytokines in vitro, among which CD3+ and CD56+ cell subsets have the best tumor-killing effect [7]. At present, the tumor-killing mechanisms of CIK cells mainly include the following:

- (1) CIK cells can recognize tumor cells and release perforin and granzyme through a variety of channels to lyse tumor cells.
- (2) CIK cells directly inhibit tumor cells by secreting cytokines (IL-2, IL-6, TNF- $\alpha$ , etc.), and indirectly cooperate with immune response to kill tumor cells.
- (3) FasL (type 11 transmembrane glycoprotein) expressed by CIK cells binds to Fas (type 1 transmembrane glycoprotein) expressed by tumor cells, thereby inducing tumor cell apoptosis. The surface of DC cells presents antigens to CD8+T cells and CD4+T cells through the antigen peptide MHCII class molecular complex on the cell surface, and stimulates the specific immune response of T cells [8]. DC cells can also secrete a specific cytokine (IL-12) which promotes T lymphocytes and NK cells to produce tumor necrosis factor, perforin and granzyme to cause tumor cell lysis. In the process of co-culture between DC and CIK cells, HLA-DR, CD40 and CD80 highly expressed on the surface of DC cells will bind to corresponding co-stimulatory molecular receptors expressed by CIK cells, and promote the secretion of IL-12, IL-2, IFN- $\gamma$  and other cytokines by DC. Also, this process will ac-

celerate the proliferation and maturation of CIK cells, and increase the number of CD3+, CD8+, CD56+ and other cell subsets in CIK cells, and improve their killing ability on tumor cells [9,10]. In addition, the level of regulatory T cells CD4+CD25+Tregs, which have strong immunosuppressive effect in CIK cells, can be significantly reduced when co-cultured with DC-CIK, thus reducing its anti-tumor inhibitory effect [11]. After receiving treatment with DC-CIK cells, the immune capacity and anti-tumor ability of patients are significantly improved, and the lifetime of patients with middle and advanced tumors are prolonged. With high survival time and safety, DC-CIK is suitable for more in-depth clinical application and promotion.



**Fig. 2 Anti-tumor function of DC-CIK after combined culture back into the patient**

### 2.3 Clinical Application and Significance

At present, many kinds of targeted therapy drugs for tumors are in clinical application, but according to actual clinical trials, there are still adverse prognostic effects of existing drugs. According to the study of Chen Xiangmei et al. [12], although the adverse prognostic reactions of the experimental group given drug therapy decreased compared with the control group given chemotherapy alone, there was a certain gap in the proportion of adverse reactions between the experimental group and the control group, there were still adverse prognostic reactions in the experimental group. In other words, the drug therapy used in the experimental group could effectively inhibit and kill tumors, but there was no way to cause a completely lethal effect to tumors. In the study of Zhang Zhongyuan et al. [13], it was found that the amount of IL-2 secreted by Th2 type and IFN- $\gamma$  secreted by Th1 type decreased under the influence of empirical anti-infective drugs and chemotherapy. Although the Th2 type which plays a role in enhancing

ing tumor was reduced, the corresponding Th1 type which plays a role in inhibiting tumor was also reduced. This indicates that the depth of current research and experiments on Th1/Th2 is not enough, and it still needs to be further studied.

In addition, DC-CIK cell immunotherapy has been clinically used and studied in a variety of cancer patients, including gastric cancer patients, liver cancer patients, urinary cancer patients, and colon cancer patients. According to the study of Zheng Shuo et al. [14], in the experimental group using DC-CIK cell immunotherapy, the content of CD8+T cells in the experimental group was lower than that in the control group, while the values of CD4+T cells and CD4+T cells/CD8+T cells were higher than that in the control group, the treatment effectiveness was higher than that in the control group, and the recurrence rate and mortality were lower than that in the control group. This indicates that DC-CIK cell immunotherapy can effectively improve the cellular immune function of patients and reduce the recurrence rate of patients. In the study of Xiao Yongping et al. [15], DC-CIK cell immunotherapy was compared with chemotherapy, and it was found that DC-CIK significantly improved the enhancement of patients' immune function. The immune function (CD3+, CD4+, CD4+/CD8+) of the experimental group after DC-CIK cell immunotherapy was higher than that of the control group, the IL-2, IFN- $\gamma$  and TNF- $\alpha$  of the experimental group were higher than that of the control group, IL-6 was lower than that of the control group, and the incidence of adverse reactions was lower than that of the control group. These results indicate that postoperative DC-CIK cell immunotherapy can improve the immune function of patients with urinary system tumor and effectively reduce the occurrence of adverse reactions. According to the study of Su Ruiliang et al. [16], postoperative chemotherapy combined with DC-CIK therapy for gastric cancer is a safe method, which can significantly inhibit the expression of tumor markers and thus inhibit the growth and metastasis of tumors. At the same time, it can also improve patients' autoimmune ability and accelerate the recovery of body functions. Restore the patient's physical condition lost due to chemotherapy, etc. Adoptive immunotherapy of DC-CIK through DC has some effect on the treatment of some tumor cells, and it does not cause adverse conditions at present [17].

### 3. Conclusion

Immune cell therapy is a new therapy for tumor treatment, including ACI and tumor-infiltrating lymphocytes. By means of ACI, the patients' own CD4+T cells and DC are extracted in vitro for targeted catalysis, which can avoid

the influence of catalytic drugs on non-target cells, and at the same time, cells can be differentiated more accurately. More precise differentiation can help cytotoxic T cells to have more powerful aids in tumor killing. Thus, a more powerful killing effect can be achieved. To meet the expectations of researchers for cancer treatment. In addition, the clinical application of ACI has a considerable degree of safety according to the available data, but the current research on the application of ACI to CD4+T cells, DC, CIK cells is still limited, and the effective prevention of postoperative adverse conditions needs to be studied, especially the adjustment of individual differences is still to be discussed. At the same time, the method of postoperative multi-stage treatment is not perfect, there are still patients whose condition deteriorates and can't be saved, so more clinical studies are needed. However, by summarizing the current research progress, this paper found that the proliferation and differentiation of CD4+T cells, DC, CIK cells through ACI and then re-transport back into the body have not yet been practiced, but have the potential to become a feasible treatment. However, there are some limitations in this study, and further experiments are needed to confirm its effectiveness. Therefore, more research on ACI on immune system cells can be done in the future.

#### Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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