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Original Research Article

Effect of anti-CD3-EGFR bispecific antibody-labeled DC-CIK immune cells on proliferation of lung cancer cells

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Abstract

Purpose: *To investigate the effect of DC-CIK immune cells labeled with anti-CD3-EGFR bispecific antibody (BSAB) on the proliferation of lung cancer (LC) cells in order to generate data useful for enhancing the therapy of LC.*
Methods: *The anti-CD3 and anti-EGFR monoclonal antibodies were labeled on the surface of DC-CIK cells using chemical coupling method. Successful preparation of anti-CD3-EGFR BSAB was determined using SDS-PAGE. Two groups of cells were used: labeled group and a DC-CIK group. The cytotoxic effect on A549 LC cells was determined in vitro, and the tumor inhibition capacity was determined in 30 nude mice with LC.*
Results: *The killing rate of EGFR-positive A549 cells in labeled group was more severe than the killing rate in DC-CIK cells ($p < 0.05$). Cellular growth rate was significantly lesser in labelled A549 LC cells than in DC-CIK group. After 2 months of treatment, nude mouse tumor size in labeled group was smaller than the tumor volume in DC-CIK group. No obvious adverse reactions (ARs) were observed in both groups.*
Conclusion: *In vitro, CD3-EGFR BSAB-labeled DC-CIK immune cells produce cytotoxic effect and inhibit the proliferation of LC cells, while in vivo studies reveal that the cells produce good therapeutic effect on LC.*

Keywords: *Anti-CD3-EGFR BSAB, DC-CIK cells, Lung cancer, Proliferation, Killing effect*

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INTRODUCTION

Lung cancer (LC) is a common disease worldwide, and it is characterized by very high rates of illness and death. The etiology of LC is very complex, but smoking is the main predisposing factor. The risk of LC in smokers is more than 10 times that in non-smokers [1]. In addition, air pollution, long-term exposure to certain chemicals, radiation and other factors are associated with LC. The symptoms of LC are not usually noticeable at the early stage of the disease, but the signs appear in the late stages [2]. The most common symptoms of LC are cough, phlegm, chest pain, shortness of breath, and hoarseness. In addition, patients may suffer from fatigue, weight loss, loss of appetite and other symptoms.

The diagnosis of LC usually requires multiple examinations [3]. The most common tests are chest X-rays, CT scans, bronchoscopy and tissue biopsies. These tests help doctors to determine the type, size, location, and spread of LC to other sites [4]. At present, the conventional treatment methods are surgical excision,

radiotherapy, and chemotherapy, among others. However, these methods have certain limitations such as poor therapeutic effect and severe toxic side effects [5]. Thus, it is crucial to develop a novel method for efficient treatment of LC.

Immunotherapy is a tumor treatment method which has attracted much attention recently. The CD3-EGFR BSAB-labeled DC-CIK immune cells are used as novel immunotherapy method which produces good therapeutic effect with minimal side effects [6]. The DC-CIK immune cells fuse onto dendritic cells (DC) and cytokine-induced killer lymphocyte (CIK) immune cells which have strong anti-tumor effect [7]. The DC-CIK immune cell therapy inhibits the growth and spread of tumor cells by enhancing immunity. Anti-CD3-EGFR BSAB is an antibody that binds both tumor cells and CIK cells, thereby further enhancing the killing effect of CIK cells [8].

Anti-CD3-EGFR BSAB is a novel tumor therapeutic drug that kills tumor cells by recognizing specific antigens on the surfaces of tumor cells [9]. This study was aimed at investigating the effect of anti-CD3-EGFR BSAB labeled DC-CIK immune cells on the proliferation of LC cells, so as to provide a new treatment method for LC. In this study, the effect of anti-CD3-EGFR BSAB-labeled DC-CIK immune cells on LC cell proliferation was determined, and the killing rate and growth rate of tumor cells in LC mice under different treatment methods were investigated. In addition, the tumor volume and incidence of ARs in different groups of mice were studied, and the antitumor effect of DC-CIK immune cells against CD3-EGFR BSAB labeling was determined, with the overall aim of providing guidance for enhancing the effectiveness of treatment of LC patients.

METHODS

Grouping of mice

Thirty 6-week-old mice were assigned to labeled and DC-CIK groups, each having 15 mice. The gender distribution and average body weight of mice in labeled group and DC-CIK mice were comparable ($p > 0.05$). The study was approved by the Animal Ethics Authority of Zhongshan Hospital Xiamen University (approval no. ZHXU2023003), and was conducted in line with the guidelines of "Principles of Laboratory Animal Care" NIH publication no. 85-23 (revised 1985).

Model development and handling procedure

The 30 mice were fed adaptively and *ad libitum* for one week, after which a mouse model of LC was established. Each mouse was inoculated in the armpit with 0.2 mL Lewis LC cells (2×10^6 cells/mouse). The injection site was pressed to prevent external leakage. After the LC cells were inoculated, tumor formation in the mice was examined daily. The long diameter (D) and short diameter (d) of the tumor were measured. Then, the tumor volume was calculated using Eq 1.

$$V = (D \times d^2)/2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

This formula assumes that the tumor had a roughly ellipsoid shape. After tumor growth, the 30 mice were assigned to 2 groups: labeled group and DC-CIK group, each with 15 animals. Mice in the labeled group were injected with DC-CIK cells which were surface-labeled with anti-CD3 and anti-EGFR monoclonal antibodies using the chemical coupling method. Mice in the DC-CIK group were injected with DC-CIK cells. Tumor volume and ARs in the two groups were monitored and analyzed.

Killing rate

Logarithmic growth phase A549 LC cells were seeded at a density of 1×10^6 cells per plate, incubated overnight with 300 µL ^{51}Cr, washed thrice, and adjusted to a cell density of 2×10^4/mL. Then, 100 µL of target cells labeled with Cr were added and plated in a 96-well plate at effector cells: target cells ratio of 80:1. The total cell volume was 200 µL. This was mixed evenly and incubated for 6 h in a 5 % CO_2 atmosphere at 37 ℃, and centrifuged. Then, the common myeloid progenitor (CMP) of 100 µL of supernatant was measured using a gamma scintillation counter. At the same time, a natural release well (target cells plus culture medium) was set up, as well as a maximum release well (target cells to which 1 % Triton was added). The concentration of A549 LC cells was 2×10^4 cells/mL, and cells were assigned to 2 groups: labeled group: EGF receptor/CD3BSAb (20 µg/mL) + DC-CK cells (20 µg/mL) + A549 LC cells; and DC-CK group: DC-CK Cells + A549 LC cells. Cytotoxicity or Lethality rate (L) was calculated using Eq 2.

$$L\ (\%) = \{(E-N)/(M-N)\}100 \quad \ldots\ldots\ldots\ldots \quad (2)$$

Where E is experimental CMP value, N is natural release CMP value and M is maximum release CMP value

Tumor volume

Tumor size was measured using an external caliper. After obtaining the longest longitudinal diameter (L) and the widest transverse diameter

(W), tumor volume (TV) was computed using Eq 3.

$TV = ½(LW^2)$ …… (3)

ARs of mice

Pleural effusion in mice were identified using ultrasonography. The fluid in the pleural space appears as a dark or light area on the ultrasound image, depending on the amount of protein and cellular content in the fluid. General signs of respiratory illness such as sneezing, difficulty in breathing, and discharge from the nose, were treated as pneumonia.

Statistical analysis

The data were recorded using Excel 2016 and processed with SPSS 20.0. Results obtained through measurements are shown as mean ± standard deviation (SD) and 2 2-group comparison was done with *t*-test. Results from enumeration are presented as percentages (%) and were compared using chi-squared (χ^2) test. Statistical significance was assumed at $p < 0.05$.

RESULTS

Killing rate of EGFR-positive A549 cells by mouse immune cells

Table 1 shows the killing rate of EGFR-positive A549 cells by mouse immune cells in the labeled group, relative to the DC-CIK group. The killing rate of EGFR-positive A549 cells by mouse immune cells in the labeled group was 85.32 %, while the killing rate in the DC-CIK group was 54.78 %. Hence, the immune cells in the labeled group demonstrated a significantly higher killing rate against EGFR-positive A549 cells than DC-CIK.

Table 1: Killing rate of EGFR-positive A549 cells by mouse immune cells (*n*=3)

Group	Killing rate (%)	*T*	*P*-value
Tag Group	85.32±2.19		
DC-CIK group	54.78±1.77	-10.931	0.001

Tumor volume in each group of mice

Table 2 displays and compares the tumor volumes of mice in both groups. Tumor volumes in the labeled and DC-CIK groups were 628 mm^3 and 793 mm^3, respectively. Therefore, labelled mice tumor size was markedly higher, when compared to that of DC-CIK mice ($p < 0.05$).

Table 2: Tumor volumes in both groups of mice (*n*=15)

Group	Tumor volume (mm^3)	*T*	*P*-value
Tag	628.13±5.49	-9.904	0.001
DC-CIK	793.80±11.36		

ARs of mice

The occurrence of pleural effusion in mice is presented in Table 3. There were 4 cases of pleural effusion in the labeled group and 3 cases in the DC-CIK group. The incidence of pleural effusion was comparable in the two groups ($p > 0.05$).

Table 3: Occurrence of pleural effusion in mice in both groups (*n*=15)

Group	Pleural effusion (*n*)		χ^2	*P*-value
	Yes	No		
Tag	4	11	0.675	0.538
DC-CIK	3	12		

The number of mice with pneumonia in each group is shown in Table 4. Three mice had pneumonia in the labeled group, while 4 mice had pneumonia in the DC-CIK group. There was no significant difference in the incidence of pneumonia between the two groups ($p > 0.05$).

Table 4: Occurrence of pneumonia in each group of mice (*n*=15)

Group	Pneumonia (*n*)		χ^2	*P*-value
	Yes	No		
Tag	3	12	0.733	0.519
DC-CIK	4	11		

DISCUSSION

Lung cancer (LC) is a common malignant tumor. However, due to the heterogeneity of tumor cells and the side effects of chemotherapy and other factors, the effect of therapy on LC is not satisfactory because the traditional therapeutic methods have limited efficacy and serious side effects. Therefore, new therapeutic methods have attracted much attention [10]. Under normal circumstances, the immune system recognizes and destroys cancerous cells, although some of the cells stave off the attack through different strategies [11]. Therefore, the purpose of immunotherapy is to activate and strengthen the immune system through various methods, so that it can recognize and destroy cancer cells. The DC-CIK immune cells are a type of immune cells frequently used in tumor therapy. These cells inhibit the growth and spread of tumor cells by enhancing the immunity of the host [12]. The DC-CIK immune cells activate the immune system to

fight tumor cells. However, anti-CD3-EGFR BSAB guides DC-CIK immune cells to the surface of tumor cells in a targeted way, thereby enhancing the therapeutic effect of these cells [13]. Moreover, anti-CD3-EGFR BSAB bind to DC-CIK immune cells and LC cells, thereby enhancing the recognition and killing of LC cells by immune cells. Anti-CD3-EGFR BSAB-labeled DC-CIK immune cells significantly inhibit the proliferation of LC cells, thereby providing a new method for LC treatment [14]. The advantages of this method are that it enhances immunity and reduces toxic side effects, and it is easily targeted at the specific antigen on the surface of tumor cells for treatment. The latest improvements in diagnostic approaches and treatment have made the treatment of LC more targeted. Therapies that inhibit tumor development by targeting specific oncogenic driver mutations may be applied to provide improved prognosis for patients [15]. The activation of mutations in epidermal growth factor receptor (EGFR) in non-small cell LC (NSCLC) is a good predictor of treatment with EGFR tyrosine kinase inhibitors (TKIs) [16]. Studies on somatic mutations in EGFR have identified a new molecular classification subgroup of NSCLC. Classical EGFR-activating mutations such as subunit deletion in exon 19 or Leu858Arg point mutations in exon 21, are linked with sensitivity to first-generation quinazoline reversible EGFR TKIs [17]. Endothelial cells related to tumors activate EGFR due to the generation of EGFR-associated ligands. Studies on the biology of the mechanism of resistance of EGFR mutant non-small cell LC may guide the development of new drugs for LC, thereby enabling patients to get more accurate and effective treatment. This study was aimed at determining the influence of DC-CIK immune cells labeled with anti-CD3-EGFR BSAB on the proliferation of LC cells. The goal was to develop new insights and methods for LC therapy. The effect of anti-CD3-EGFR BSAB-labeled DC-CIK immune cells on LC cell multiplication was unraveled, and the killing rate and growth rate of tumor cells in labeled and CD-CIK groups of LC mice were measured, in addition to comparison of the tumor volume and the occurrence of ARs between the two groups of mice. The results obtained revealed that the killing rates of labeled mice and DC-CIK groups with EGFR-positive A549 cells were 85.32 and 54.78 %, respectively. The killing rate of EGFR-positive A549 cells by immune cells was higher in the DC-CIK group than in the labeled group. The growth rates of A549 LC cells in mice in the labeled and DC-CIK groups were 81.22 and 102.78 %, respectively, showing a significant difference. Labeled mice had a markedly smaller tumor size (628 mm^3) than DC-CIK mice (793 mm^3). The labeled group had 4 instances of pleural effusion, 3 instances of pneumonia, 2 cases of atelectasis, 2 cases of emphysema, and 1 case of pulmonary embolism. In DC-CIK group, 3 mice had pleural effusion, 4 mice had pneumonia, 2 mice showed atelectasis, 1 mouse had emphysema, in addition to 2 cases of pulmonary embolism. There were significant differences in cases of ARs between the DC-CIK and the labeled groups. This study has established that anti-CD3-EGFR BSAB-labeled DC-CIK immune cells exert an inhibitory effect on LC cell proliferation. This finding has high therapeutic value and application potential.

Limitations of this study

This work has some shortcomings. For example, the experimental sample size was small. Therefore, there is need for expansion of the sample size in subsequent studies so as to improve the reliability of experimental results. In addition, this work determined the effect of DC-CIK immune cells on LC cells only at the cellular level. There is need for animal experiments and clinical trials.

CONCLUSION

Anti-CD3-EGFR BSAB-labeled DC-CIK immune cells significantly inhibit the proliferation of LC cells, thereby providing a new method for LC treatment. This method has the advantages of specificity and low side effects, but further studies are needed to confirm its clinical effect.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Minjie Li designed the study, supervised the data collection, and analyzed the data. Yuan Zhong interpreted the data and prepared the manuscript for publication. Minjie Li supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

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