

Original Article

Expression of CD105 and CD4⁺CD25⁺Foxp3⁺ Treg in peripheral blood of patients with non-small cell lung cancer undergoing Endostar plus chemotherapy and its significance

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Abstract: *Objective:* This study was designed to observe changes in peripheral blood CD105 and CD4⁺CD25⁺Foxp3⁺ regulator T cells (Treg) in patients with non-small cell lung cancer (NSCLC) who underwent Endostar plus chemotherapy, and to evaluate the relationship between observation indexes and clinical therapeutic effects. *Methods:* A total of 57 NSCLC patients were included into this study and randomly divided into two groups: observation group ($n=29$, Endostar combined with chemotherapy), and control group ($n=28$, chemotherapy alone). Serum CD105 was detected by enzyme-linked immunosorbent assay (ELISA), and CD4⁺CD25⁺Foxp3⁺ Treg was detected by fluorescence activated cell sorting (FACS). *Results:* The clinical effective rate was 72.41% (21/29) in the observation group and 42.86% (12/28) in the control group, and the difference in effective rate between these two groups was statistically significant ($P<0.05$). The levels of CD105 and CD4⁺CD25⁺Foxp3⁺ Treg in peripheral blood were significantly lower in these two groups after treatment ($P<0.05$). After treatment, the decrease in CD105 and CD4⁺CD25⁺Foxp3⁺ Treg levels in peripheral blood was more significant in the observation group than that in the control group ($P<0.05$). In the observation group, after four courses of chemotherapy, peripheral blood CD105 and CD4⁺CD25⁺Foxp3⁺ Treg levels were positively correlated with the curative effect; while these were not correlated with the curative effect in the control group. *Conclusion:* Endostar combined with chemotherapy has a high therapeutic effect in the treatment of NSCLC, and CD105 and CD4⁺CD25⁺Foxp3⁺ Treg may serve as new indicators for predicting its therapeutic effect.

Keywords: Endostar, chemotherapy, non-small cell lung cancer, CD105, CD4⁺CD25⁺Foxp3⁺ Treg

Introduction

The occurrence and development of tumors are closely related to angiogenesis, and are also associated with the escape of tumor cells from immune surveillance. Endostar is a self-developed new recombinant human endostatin (rh-endostatin) in China, which has a multiple-target anti-tumor angiogenesis effect [1]. The anti-tumor angiogenesis effect of Endostar mainly manifest as that: (1) Specifically exerting on endothelial cells in new blood vessels and induces apoptosis; (2) Regulating the expression of vascular endothelial growth factor and the activity of proteolytic enzyme on the surface of tumor cells, causing tumor to shrink or hibernate. Because its mechanism is entirely different from that of cytotoxic chemotherapeu-

tic drugs, Endostar combined with direct cytotoxic chemotherapeutic drugs has become an effective pattern for tumor treatment. In 2005, Endostar combined with chemotherapy is approved by the Food and Drug Administration of China to be used in first-line chemotherapy for non-small cell lung cancer. Due to its good effectiveness and safety, it has been widely used in the treatments of gastrointestinal tumors, ovarian cancers and breast cancers now [2-5]. CD105, also known as endoglin, is highly and specifically expressed in endothelial cells in a variety of tumor tissues involved in the formation of tumor blood vessels; and is closely related to the occurrence and development of tumors [6]. Studies have revealed that, CD105 was preferentially expressed in the activated endothelial cells in the new blood vessels in

tumor tissues, and is a more accurate indicator to measure endothelial cell proliferation currently [5-10]. Therefore, in this study, this index was chosen to evaluate antiangiogenic effects of Endostar. Tregs is a T cell subset with regulatory function, can inhibit the immune response, and plays an important role in maintaining the stability of immune function, inhibiting tolerance and tumor immunity. As a specific molecular marker of regulatory T cells, Foxp3 transcription factor plays an important role in regulating the development of regulatory T cells [11, 12]. CD4⁺CD25⁺Foxp3⁺ Treg can maintain immune tolerance in patients with cancer. Hence, it is of great significance in anti-tumor therapy. A study has reported that, change in the positive rate of CD4⁺CD25⁺Foxp3⁺ Treg cells before and after chemotherapy was related to the therapeutic effect of chemotherapy. But few related literatures have focused on this. A present study has revealed that in addition to inhibiting angiogenesis, Endostar also has important effects on the expression of many genes [12]. This study aims to investigate the effects of Endostar combined with chemotherapy on a tumor micro-environment in patients with lung cancer. Details are reported as follows.

Materials and methods

General information

From January 2013 to December 2015, a total of 57 patients with advanced non-small cell lung cancer (NSCLC), who were primarily treated in our department, was enrolled into this study. The age of these patients ranged from 40 to 69 years old, with a median age of 58 years old. Among these patients, 25 patients were in stage IIIA, 18 patients were in stage IIIB, and 14 patients were in stage IV. Furthermore, among the 57 patients, 41 patients had adenocarcinoma, 13 patients had squamous cell carcinoma, and three patients had sarcomatoid carcinoma. All diagnoses were confirmed by pathology and imaging. Before the treatment, routine blood test, as well as liver and kidney function tests, revealed no obvious abnormalities; and their Karnofsky scores were ≥ 70 points. These patients were randomly divided into two groups: observation group ($n=29$), and control group ($n=28$). All patients provided a signed informed consent. Exclusion criteria: Patients who were combined with infectious diseases, autoimmune diseases and

allergic diseases that have been verified to lead to changes in CD4⁺CD25⁺ Treg level were excluded.

Therapeutic methods

In the observation group, the "paclitaxel + cisplatin + Endostar" regimen was adopted: 135 mg/m² of paclitaxel, administered on day one; 75 mg/m² of cisplatin, administered on day two; 15 mg of Endostar, administered on days 1-14. Three weeks of treatment was considered as one course, and delayed treatment due to adverse reactions should not be more than one week. In the control group, the "paclitaxel + cisplatin" regimen was adopted. The doses were the same as those in the observation group. CT was performed on each of the two courses to evaluate for efficacy. For patients who had an invalid outcome, chemotherapy regimen was altered. All patients received at least four courses of chemotherapy.

Main instruments and reagents

The RT6000 enzyme micro-plate reader was purchased from Shenzhen Rayto. The S-CD105 kit was purchased from the United States of America. Fluorescence activated cell sorting (FACS) Calibur flow cytometry, CD4-FITC (fluorescein isothiocyanate-labeled CD4), CD25-APC (allophycocyanin-labeled CD25), Foxp3-PE and the isotype control, and Human Foxp3 Buffer Set were all obtained from Becton, Dickinson and Company (USA).

Detection methods

Serum index acquisition: In the morning two days before chemotherapy and at the end of the second and fourth course of chemotherapy, 3 ml of venous blood was withdrawn from each subject. The collected blood was placed at room temperature for four hours, centrifuged at 3,000 rpm for 10 minutes, and 1 ml of serum was isolated. The serum was placed in a 1.5-ml polypropylene glycol ester test tube, and stored at -70°C in a refrigerator. Serum soluble CD105 levels were measured in batches by enzyme-linked immunosorbent assay (ELISA; Genzyme, USA). The experiment was carried out strictly according to the instruction manual. Cytological index detection: Fasting venous blood (3 ml, each subject) was withdrawn two days before treatment and at the end of the fourth course of chemotherapy, respectively. The blood was

Serum soluble CD105 and CD4⁺CD25⁺Foxp3⁺ Treg

Table 1. Comparison of clinical curative effect

Groups	Cases	CR	PR	SD	PD	X ²
The control group	28	2	10	13	3	5.105
The observation group	29	3	18	6	2	

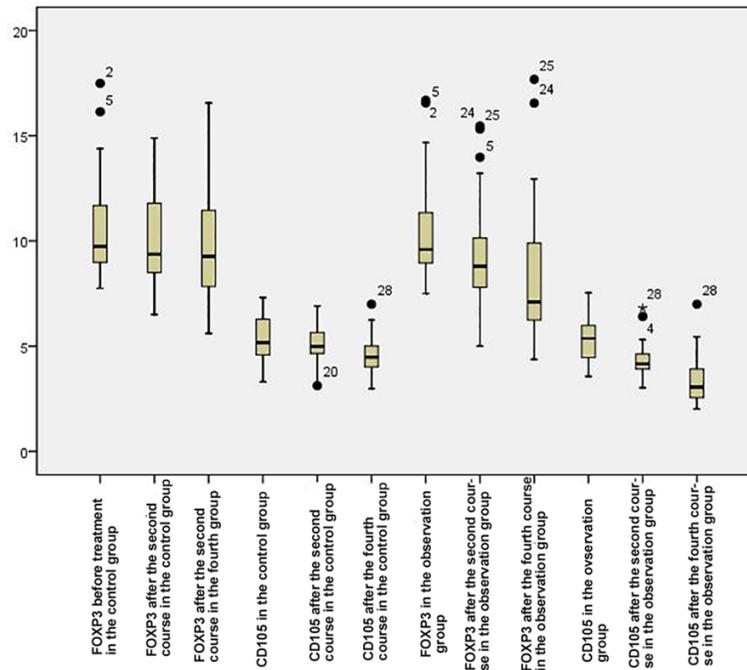


Figure 1. Comparison of CD4⁺CD25⁺Foxp3⁺ Treg and CD105 levels in peripheral blood before and after treatment.

added with ethylenediamine tetraacetic acid dipotassium salt (EDTA-K2) for anticoagulation, and the cytological indexes were detected using CytoFLEX flow cytometry (Beckman Coulter, USA), FACS Calibur flow cytometry, CD4-FITC, CD25-APC, Foxp3-PE and the isotype control, and the Human Foxp3 Buffer Set. All the above instruments and reagents were obtained from Becton, Dickinson and Company (USA).

Evaluation of curative effects and adverse reactions

With reference to the entity tumor curative effect evaluation standard of the World Health Organization (WHO), the curative effects were classified into five levels: pathological complete remission (pCR), clinical complete remission (cCR), clinical partial remission (PR), stable disease (SD), and disease progression (PD). Effective rate = (CR + PR)/number of cases × 100%.

Statistics processing

Data were statistically analyzed using the statistical software SPSS 17.0. Measurement data were expressed as mean ± standard deviation (SD), and evaluated using analysis of variance. Count data were evaluated using X² test. Correlation analysis was evaluated using the nonparametric test. *P*<0.05 was considered statistically significant.

Results

Comparison of clinical curative effect

The effective rate was 72.41% (21/29) in the observation group, and 42.86% (12/28) in the control group, and the difference in effective rate between these two groups was statistically significant (*P*<0.05, **Table 1**).

Comparison of CD105 levels in peripheral blood before and after treatment (Figure 1)

CD105 levels in peripheral blood were significantly lower in patients after treatment in these two groups (*P*<0.05). After treatment, the decrease in CD105 levels in peripheral blood was more significant in the observation group than that in the control group (*P*<0.05, **Table 2**).

Comparison of the CD4⁺CD25⁺Foxp3⁺ Treg levels in peripheral blood before and after treatment (Figure 1)

CD4⁺CD25⁺Foxp3⁺ Treg levels in peripheral blood were significantly lower after treatment in these two groups (*P*<0.05). After treatment, the decrease in CD4⁺CD25⁺Foxp3⁺ Treg level in peripheral blood was more significant in the observation group than that in the control group (*P*<0.05, **Table 3**).

Relationship between peripheral blood CD105 levels and curative effects

In the observation group, peripheral blood CD105 levels was not correlated with the cura-

Serum soluble CD105 and CD4⁺CD25⁺Foxp3⁺ Treg

Table 2. Comparison of CD105 levels in peripheral blood before and after treatment (x±s)

Groups	Time	CD105
The control group	Before treatment	5.31±1.10
	After treatment	4.65±1.10*
The observation group	Before treatment	5.28±1.05
	After treatment	3.29±1.09*,#

Note: *Compared with before treatment, $P<0.05$; #After treatment, compared the observation group with the control group, $P<0.05$.

Table 3. Comparison of the CD4⁺CD25⁺Foxp3⁺ Treg levels in peripheral blood before and after treatment (x±s)

Groups	Time	CD4 ⁺ CD25 ⁺ Foxp3 ⁺ /CD4 ⁺
The control group	Before treatment	10.64±2.40
	After treatment	9.97±2.99*
The observation group	Before treatment	10.49±2.48
	After treatment	8.31±3.19*,#

Note: *Compared with before treatment, $P<0.05$; #After treatment, compared the observation group with the control group, $P<0.05$.

tive effect after two courses of chemotherapy, but positively correlated with the curative effect ($r=0.553$) after four courses of chemotherapy. Furthermore, peripheral blood CD105 levels in patients in the control group was not correlated to the curative effect ($r=0.162$) after treatment.

Relationship between peripheral blood CD4⁺CD25⁺Foxp3⁺ Treg levels and curative effects

In the observation group, peripheral blood CD4⁺CD25⁺Foxp3⁺ Treg levels were not correlated with the curative effects after two courses of chemotherapy, but was positively correlated with curative efficacy ($r=0.494$) after four courses of chemotherapy. The CD4⁺CD25⁺Foxp3⁺ Treg level in peripheral blood in patients in the control group after treatment was not correlated to the curative effect ($r=0.032$).

Discussion

In recent years, the incidence and mortality of lung cancer has continuously increased year by year. A previous study revealed that the number of micrangia in a tumor was closely correlated to the growth and metastasis of the tumor [13]. CD105 is a component of transforming growth factor- β (TGF- β) receptor complexes, which

has two isomers: L-CD105 and S-CD105. The latter is the soluble component of CD105. Studies have revealed that CD105 was preferentially expressed in endothelial cells that have been activated and are involved in the formation of new blood vessels in tumor tissues, but was weakly expressed or not expressed in normal tissue vessels. At present, it is a relatively accurate indicator for measuring endothelial cell proliferation [7-10]. Yiqin Ai *et al.* [14] reported that serum CD105 levels in patients with nasopharyngeal carcinoma was higher than that in normal subjects, and was significantly decreased after radiotherapy. They considered that CD105 can serve as a new indicator for the detection of conditions of diseases in patients with nasopharyngeal carcinoma before and after treatment.

Chen Li *et al.* [15] revealed that serum soluble CD105 levels in patients with NSCLC was significantly higher than that in the control group, and the level of serum soluble CD105 in patients after surgery was significantly lower than that before surgery. Furthermore, Xianxiong Zou *et al.* [12] reported that CD105 and CD34 protein expression levels were significantly lower in stomach cancer patients who underwent the combined treatment of Endostar and chemotherapy, when compared with patients who underwent chemotherapy alone; and the differences in metastasis- and apoptosis-related indicators between these two groups were statistically significant. They considered that Endostar combined with chemotherapy could effectively inhibit tumor vascular formation, invasion and metastasis; promoting tumor cell apoptosis.

The occurrence of malignant tumors is also correlated to the escape of tumor cells from immune surveillance. Treg is a T cell subset with regulatory function that can inhibit immune response and maintain the stability of its own immune function under normal conditions [11, 16]. Transcription factor Foxp3 is specifically expressed in Tregs. Among these, suppressor T cells, mainly, CD4⁺CD25⁺Foxp3⁺ Treg cells, play an important role in tumor immunity [17-19]. Previous studies [20-22] have revealed that in

the occurrence and development of malignant tumors, with the increase in the number and function of CD4⁺CD25⁺Foxp3⁺ Tregs, an immune escape environment was formed and provided to tumors. Rong Yu *et al.* [23] reported that the change in the positive rate of CD4⁺CD25⁺Foxp3⁺ Tregs in peripheral blood in patients with advanced ovarian cancer before and after chemotherapy was correlated to the therapeutic effect of chemotherapy, which can serve as a predictor for predicting the curative effect of chemotherapy.

The results of this study revealed that the clinical effective rate was significantly higher in the observation group than in the control group (72.41% vs. 42.86%).

In this study, the levels of CD105 and CD4⁺CD25⁺Foxp3⁺ Treg in the peripheral blood in patients in the observation and control groups after treatment were compared with the levels before treatment, although the extent of decrease were not very significant, but the differences were just statistically significant. Furthermore, the extent of decrease in the observation group was more significant than in the control group. This proves that, Endostar may induce the phenomenon through an unknown mechanism. Endostar may affect tumor microenvironment and change immune function by inhibiting angiogenesis. However, this still needs further study to confirm.

In this study, peripheral blood CD105 and CD4⁺CD25⁺Foxp3⁺ Treg levels were correlated with the curative effect in the observation group. This was consistent with the results of a previous study [23]. In the observation group, peripheral blood CD105 and CD4⁺CD25⁺Foxp3⁺ Treg levels were not correlated with the curative effect after two courses of chemotherapy, but there was a correlation between these two after four course of chemotherapy. This suggests that Endostar may improve anti-tumor curative efficacy through the persisting action of the long-term treatment course.

Anti-angiogenesis drugs combined with chemotherapy has become an effective pattern of cancer treatment. However, a number of studies have suggested that the characteristics of this kind of drugs inhibited the growth of tumor blood vessels. Furthermore, its curative effects become more significant with the increase in

the number of courses, but these could not rapidly reduce the volume of tumors in a short period of time [20, 24]. Due to delay and non-synchronization, routine imaging evaluation system based on tumor volume changes cannot reflect the curative efficacy of anti-angiogenesis drugs in real time. Therefore, searching for an effective evaluation index has become an important research direction. This study revealed that in patients who underwent Endostar combined with chemotherapy, peripheral blood CD105 level was correlated to curative efficacy. This suggests that CD105 may serve as an indicator to monitor the curative effect of anti-angiogenesis drugs. In this study, in patients who underwent chemotherapy alone, a relationship between CD105 and CD4⁺CD25⁺Foxp3⁺ Treg levels and the curative effect was not found. These needs to be further observed in large-sample studies.

Disclosure of conflict of interest

None.

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