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Effect of dendritic cell immunotherapy on distribution of dendritic cell subsets in non-small cell lung cancer

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Abstract. The effect of dendritic cell (DC) immunotherapy on non-small cell lung cancer (NSCLC) and its influence on the distribution of DC subsets were studied. Peripheral blood was drawn from 55 patients, and DCs were cultured *in vitro* and injected into the patients three times. The changes in DC subsets in NSCLC patients before treatment and after three treatments were observed using a flow cytometer, and the difference in DC subsets between patients and healthy controls was compared. DC subsets in lung cancer tissues, para-carcinoma tissues and normal tissues were analyzed by indirect immunofluorescence and laser scanning confocal microscope (LSCM). The BDCA-1$^+$ DC1 and BDCA-3$^+$ DC2 in lung cancer tissues were significantly increased compared with those in para-carcinoma tissues and normal tissues ($P<0.05$). The number of DC1 and DC2 in para-carcinoma tissues were increased compared with those in normal tissues ($P<0.05$). The ratio of DC1 in peripheral blood in the normal control group was obviously higher than that in NSCLC patients ($P<0.01$). There were significant differences in DC1 and DC1/DC2 ratio in NSCLC patients with different tumor staging, and there were also obvious differences in patients with a different Karnofsky performance status (KPS) score. Moreover, compared with those before treatment, DC1 and DC1/DC2 ratio were significantly increased after three treatments, and there was a significant difference in the comparison of DC1/DC2 ratio between the NSCLC patients with survival time greater than and less than one year. The immune function of NSCLC patients was improved after DC immunotherapy. The survival time of NSCLC patients was closely associated with the DC1/DC2 ratio in peripheral blood. The detection of DC subsets in peripheral blood can help clinicians understand the immune function of NSCLC patients and provide a basis for the clinical judgment of prognosis of NSCLC patients.

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Introduction

Lung cancer is a malignant tumor with high morbidity and mortality rates that causes serious harm to patients and their families. With the increasing deterioration of the environment, the morbidity and mortality rates of lung cancer have shown a gradual upward trend (1,2). Some scholars suggested that the immune function of patients becomes abnormal in the occurrence and development of lung cancer (3).

The dendritic cell (DC) is the most powerful antigen-presenting cell (APC) in the body, and plays a key role in immunological stress and immunoregulation of the body (4,5). DC is a useful cell in tumor biological immunotherapy. Most scholars in China and worldwide hope to enhance the effect of immunotherapy by basis for the clinical judgment of prognosis improving the immune function and number of autologous DCs in patients, but the *in vivo* effect of DC immunotherapy is currently still significantly inferior to the *in vitro* effect. It has been confirmed that the number of DCs in the tumor-bearing host is reduced and it is defective with regard to its function compared with normal DCs (6,7). This result is universally recognized as one of the important mechanisms of tumors evading immune surveillance in the body. Studies have shown that the changes in DC1, DC2 and DC1/DC2 ratio in peripheral blood can well reflect the current immune function status of the body (8-10).

Therefore, the distribution and number of DC subsets in peripheral blood in patients with non-small cell lung cancer (NSCLC) after DC immunotherapy were detected in the present study to investigate the clinical significance of DC subset detection.

Subjects and methods

Subjects. A total of 55 patients, aged 19-77 years, who were diagnosed as NSCLC from January, 2016 to January, 2017 were selected. Exclusion criteria for the study were: Patients with dysfunction in heart, liver, kidney or hematopoietic function; child patients, pregnant women or mentally-ill patients; and patients without clear pathological diagnosis, or whose estimated survival was less than six months. Eighteen healthy

subjects, aged 22-75 years, underwent physical examination and were selected as normal controls.

All the subjects signed informed consent, and this study was approved by the Dezhou Hospital Ethics Committee (Dezhou, China).

Reagents and instruments. The reagents and instruments used in the study were: BD dendritic cell flow cytometry reagent (Guangzhou Shuoheng Biotechnology Co., Ltd., Guangzhou, China); mouse mAb-BDCA-1 and mouse mAb-BDCA-3 (Shanghai Runwelltac Industrial Co., Ltd., Shanghai, China) and the corresponding secondary antibody and TO-PRO-3 (Shanghai Jiwei Biotechnology Co., Ltd., Shanghai, China); FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA); laser scanning confocal microscope (LSCM) (Bio-Rad, Hercules, CA, USA) and small desktop high-speed centrifuge (BD Biosciences).

Detection using flow cytometer. In strict accordance with the instructions of BDDC flow cytometry reagent, DC flow cytometry reagent was added into the five heparin anticoagulant tubes, each containing 100 μl peripheral blood, and the mixture was shaken gently and evenly, followed by reaction at room temperature in the dark for 15 min. Then, 2-3 ml hemolysin was added and the mixture was shaken gently and evenly for splitting for 10 min, followed by centrifugation at 1,080 x g for 5 min. The supernatant was discarded, and the mixture was washed with phosphate-buffered saline (PBS) three times, added with 300 μl PBS and gently agitated in the dark at 4˚C. The DC subsets were detected using the FACSCalibur flow cytometer. Cells ($5x10^4$) were collected into tubes. The threshold value was set in FSC to eliminate the interference of debris on the results. CellQuest software was used to obtain and analyze the HLA-DR positive, Lin-1 weakly positive and negative cell groups in the HLA-DR/Lin-1 point diagram. The results were recorded using the percentage of positive cells in CD11c (DC1) and CD123 (DC2) fluorescent antibody staining, while the DC1/DC2 ratio was calculated.

Detection of the number of DC subsets in lung cancer, para-carcinoma and normal lung tissues. The lung cancer, para-carcinoma (~2 cm around the cancer) and normal lung tissues were snap frozen and stored in liquid nitrogen (-196˚C). The above three kinds of tissues stored in the liquid nitrogen were taken, washed with the pre-cooled PBS and placed in the blocking solution containing 10% goat serum for 45 min. Then, the tissues were placed in the diluent with donkey anti-goat BDCA-1 and BDCA-3 polyclonal antibodies (dilution, 1:20; cat. nos. AF5910 and AF3894;) for incubation at 4˚C overnight. Tissues were washed with pre-cooled PBS and goat anti-mouse secondary polyclonal antibody (dilution, 1:400; cat. no. AF109) was used for incubation at room temperature for 2 h, followed by re-staining via TO-PRO-3 and sealing.

LSCM detection. LSCM was equipped with three kinds of ion lasers, namely argon-krypton (480 nm), chlorine-neon (543 nm) and helium-neon (633 nm). Using the objective and water lens (x20), confocal images were taken on the z-axis of the slide at an interval of 500 nm, and the visual field with the most DCs was the field observed. The number of DC1- and DC2-positive cells were observed and counted by two individuals, and the average count was taken; the count difference between the two individuals was not >20%.

Table I. Comparisons of DC subsets in peripheral blood between NSCLC patients and normal controls (mean ± SD).

Groups	No.	DC1	DC2	DC1/DC2
NSCLC	55	0.61±0.33[a]	0.28±0.13	3.15±2.97
Control	18	0.79±0.24	0.26±0.11	3.94±1.89
t-value		2.884	0.537	-1.526
P-value		0.008	0.745	0.183

[a]P<0.01 compared with normal control group. DC, dendritic cell.

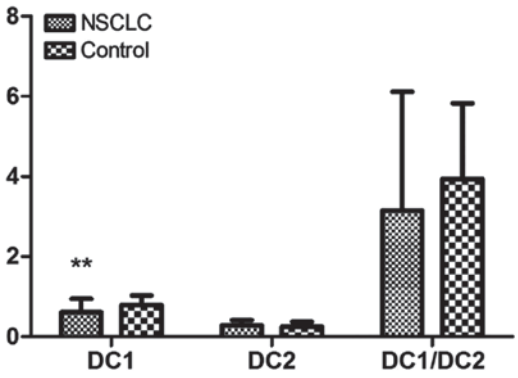

Figure 1. Dendritic cell (DC) subsets in peripheral blood in non-small cell lung cancer (NSCLC) patients and normal controls. **P<0.01, compared with control group.

Statistical analysis. SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used. Measurement data are presented as mean ± SD. The ANOVA was used for the intergroup comparison and LSD test was used as post hoc test. The Chi-square test was used for the comparisons of enumeration data or rate; α=0.05.

Results

Comparisons of DC subsets in peripheral blood between NSCLC patients and normal controls. The expression of DC1 in peripheral blood in NSCLC patients was significantly decreased compared with that in the normal control group, and the difference was statistically significant (P<0.01). The expression of DC2 did not change significantly in the NSCLC and normal control groups. Compared with that in the normal control group, the DC1/DC2 ratio in the NSCLC group was lower, but there was no statistically significant difference between the groups (P>0.05) (Table I and Fig. 1).

Relationship between DC subsets and clinical features in NSCLC group. There were no statistically significant differences in the sex, age, pathological type, CEA and other DC subsets for the 55 NSCLC patients (P>0.05). There were statistically significant differences in DC1 and DC1/DC2 ratio in NSCLC patients with different tumor staging (P=0.029

Table II. Relationship between DC subsets and clinical features in NSCLC group.

Characteristics	No.	DC1	P-value	DC2	P-value	DC1/DC2	P-value
Sex							
Male	34	0.58±0.33	0.336	0.24±0.18	0.295	2.96±2.05	0.903
Female	21	0.51±0.24		0.21±0.13		2.79±1.88	
Age (years)							
<70	40	0.62±0.34	0.103	0.25±0.13	0.135	2.67±1.75	0.194
≥70	15	0.48±0.29		0.21±0.15		3.86±3.27	
Staging							
Early	18	0.77±0.28	0.029[a]	0.26±0.15	0.894	4.47±3.01	0.001[b]
Late	37	0.52±0.23		0.25±0.13		2.56±1.73	
Pathology							
Adenocarcinoma	36	0.59±0.32	0.406	0.27±0.12	0.184	2.89±2.15	0.243
Squamous carcinoma	15	0.63±0.36		0.31±0.19		2.46±1.63	
Others	4	0.74±0.44		0.24±0.15		4.41±2.74	
KPS							
<60	20	0.48±0.31	0.025[a]	0.26±0.12	0.184	2.62±1.84	0.402
≥60	35	0.72±0.38		0.29±0.18		3.14±3.08	
CEA							
Normal	26	0.68±0.41	0.091	0.29±0.18	0.724	3.62±2.96	0.056
Abnormal	29	0.57±0.38		0.26±0.13		2.38±1.44	

[a]P<0.05 and [b]P<0.01. NSCLC, non-small cell lung cancer; KPS, Karnofsky performance status; DC, dendritic cell.

Table III. Comparisons of DC subsets in peripheral blood before and after three DC treatments.

Groups	No.	DC1	DC2	DC1/DC2
Control	18	0.79±0.24	0.26±0.11	3.94±1.89
Before treatment	55	0.61±0.33	0.28±0.13	3.15±2.97
After treatment	55	0.72±0.26[a]	0.27±0.12	3.54±2.38[b]

[a]P<0.01 and [b]P<0.05, compared with that before treatment. DC, dendritic cell.

Table IV. Relationship between peripheral blood subsets and prognosis of NSCLC patients.

	Survival time			
Groups	≥1 year	<1 year	t-value	P-value
DC subset				
DC1	0.71±0.33	0.65±0.36	-1.488	0.122
DC2	0.29±0.17	0.27±0.15	-0.174	0.916
DC1/DC2	2.94±1.24	1.73±1.27	-2.546	0.019

NSCLC, non-small cell lung cancer; DC, dendritic cell.

and P=0.001), while there were statistically significant differences in patients with a different Karnofsky performance status (KPS) scores (P=0.025) (Table II).

Comparisons of DC subsets in peripheral blood before and after three DC treatments. In NSCLC, DC1 expression was low, and the immune function of body was decreased significantly. The DC1 content and DC1/DC2 ratio after three DC treatments were significantly increased compared with those before treatment (Table III).

Relationship between peripheral blood subsets and prognosis of NSCLC patients. After three DC treatments, 49 NSCLC patients were followed up for >1 year and 32 patients were lost to follow-up or died, and the 1-year survival rate was 34.7%. There were no statistically significant differences in the comparisons of DC1 and DC2, limited by 1-year survival time (P=0.122 and P=0.916), but there was statistically significant difference in the DC1/DC2 ratio between patients with the survival time of greater than and less than one year (P=0.019) (Table IV).

The number of DC subsets in lung cancer, para-carcinoma and normal lung tissues. The number of DC1 in para-carcinoma and normal lung tissues was significantly lower than that in lung cancer tissues, and the difference was statistically significant (P<0.05). The number of DC2 in para-carcinoma and normal lung tissues was also evidently lower than that in lung cancer tissues, and the difference was statistically significant (P<0.05). Compared with those in normal lung tissues, the number of DC1 and DC2 in para-carcinoma tissues was increased significantly, and the differences were statistically significant (P<0.05) (Fig. 2).

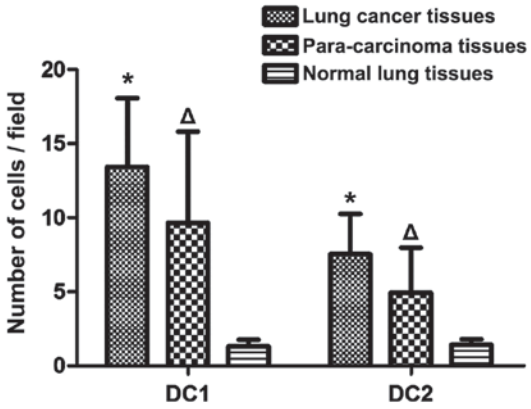

Figure 2. The number of dendritic cell (DC) subsets in lung cancer, para-carcinoma and normal lung tissues. *P<0.05 compared with that in para-carcinoma and normal tissues; ΔP<0.05 compared with that in normal tissues.

Discussion

With the aggravation of environmental pollution, the morbidity and mortality rates of lung cancer have been on the increase, seriously threatening human health (11). At present, the main types of therapy are radiotherapy, chemotherapy and biological immunotherapy. DC is the most powerful APC known currently, playing an important role in triggering the primary immune response in the body for the first time, which can effectively control tumor cell growth and metastasis (12). There are two sources of DC in the body. One is the myeloid-derived DC from bone marrow $CD34^+$ cells, which can stimulate the T-helper cell 1; consequently, it is also known as DC1. Such a cell is the most important source of DC cells at present, which can induce the secretion of a variety of cytokines from T cells in the body, thus responding to Th1 and finally triggering immune rejection in the body. The other one is lymphocytic DC derived from the thymus, which can stimulate the T-helper cell 2. Consequently, it is also known as DC2. After contacting with the tumor cells in the body, it immediately secretes a variety of cytokines, including interferon-α, triggering Th2 immune response, which is closely related to the immune tolerance response in the body (13,14).

In the present study, the number of DCs and its subsets were detected via indirect immunofluorescence and LSCM. The results of the statistical analysis revealed that DC1 and DC2 in lung cancer tissues were significantly increased compared with those in para-carcinoma and normal lung tissues ($P<0.05$), and the number of DC1 and DC2 in para-carcinoma tissues was increased compared with that in the normal tissues ($P<0.05$). The expression of DC subsets in peripheral blood in NSCLC patients before and after DC immunotherapy were detected using a flow cytometer. The results revealed that the ratio of DC1 in peripheral blood in the normal control group was significantly higher than that in NSCLC patients ($P<0.01$). There were significant differences in DC1 and DC1/DC2 ratio in NSCLC patients with different tumor staging, and they also had obvious differences in patients with different KPS scores. Compared with those before treatment, DC1 and DC1/DC2 were significantly increased after three treatments, and there was a significant difference in DC1/DC2 between NSCLC patients with the survival time greater than and less than one year.

It is currently known that DC1 in peripheral blood is reduced in a variety of malignant tumors, such as pancreatic, colorectal and liver cancer (15-17). However, there are also exceptions, such as myeloma (18). Previous findings have shown that the number of DC1 in peripheral blood does not change significantly in myeloma patients. Additionally, the increase of expression levels of various cytokines in serum is closely related to the decrease in DC in peripheral blood (18). The experiment further confirmed that the supernatant of tumor cells cultured *in vitro* can inhibit the differentiation of DC *in vitro*, and it is speculated that the tumor cells secrete some cytokines, thus inhibiting the differentiation of DC precursor *in vivo* (19-21). This may be one of the reasons why the anti-tumor effect of DC therapy *in vivo* is less than that *in vitro*.

In conclusion, the immune function of NSCLC patients is improved after DC immunotherapy. The survival time of NSCLC patients is closely related to the DC1/DC2 ratio in peripheral blood. The detection of DC subsets in peripheral blood can help clinicians understand the immune function of NSCLC patients and provide a basis for the clinical judgment of prognosis of NSCLC patients.

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Availability of data and materials

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

Authors' contributions

ZY contributed to analysis using flow cytometry and wrote the manuscript. FD performed LSCM detection. LM performed and analysed detection of the number of DC subsets. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the Dezhou People's Hospital Ethics Committee (Dezhou, China). All the subjects signed informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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