Review Article

Role of Dendritic Cells in Neoadjuvant Immuno combination Chemotherapy for Non-small Cell Lung Cancer

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Abstract

Dendritic cells (DCs) are the most powerful antigen-presenting cells (APCs) in the body and play a crucial role in immune stress and immunomodulation. The aim of this study was to analyze the molecular phenotypic changes in DC cells in non-small cell lung cancer (NSCLC) patients after neoadjuvant immunocombination chemotherapy to explore potential biomarkers associated with response to chemotherapy. Tumor tissues (Tumor) from 32 patients were collected in the study, and the DC cell subpopulation occupancy between the untreated group (treatment naïve, TN), pathological remission group (MPR), and non-remission group (non-MPR) was examined using flow cytometry. The results showed that in tumor tissues, the percentage of DCs was significantly lower in the MPR group than in the TN group, and significantly higher in the non-MPR group, and the percentage of DCs was significantly higher in the non-MPR group than in the MPR group (P<0.05). These results suggest that the changes in the distribution of DCs may be closely related to the response to neoadjuvant immune-combination chemotherapy, providing an important basis for an in-depth understanding of the immune mechanism in lung cancer treatment and laying the foundation for the development of potential biomarkers.

More than 80% of lung cancer patients have non-small cell lung cancer (NSCLC), yet the five-year relative survival rate is low [1]. As a result, lung cancer is one of the leading causes of cancer-related deaths in both men and women worldwide. In recent years, with the emergence of new therapies such as targeted therapy and immunotherapy, it has brought a new dawn of treatment to some patients [2]. Neoadjuvant immune checkpoint inhibitors (ICIs) have improved the clinical benefit of some lung cancer patients, but there is still a lack of precise biomarkers to screen out those who can benefit [3].

DCs are the most powerful antigen-presenting cells (APCs) in the body, and as a key component of the immune system, DCs play a vital role in the initiation and regulation of the immune response [4]. They are the most powerful antigen-presenting cells in the immune system, capable of ingesting, processing, and presenting antigens, activating naïve T cells, and initiating adaptive immune responses [5]. With the deepening of research, more characteristics and functions of DC cells have been gradually revealed, bringing new opportunities for the treatment and prevention of diseases. Studies have shown that factors such as the number and functional status of DCs and their presentation efficiency to lung cancer-related antigens are closely related to the efficacy of lung cancer treatment [6]. It has been reported in the literature that DCs have made some progress in the therapeutic efficacy of lung cancer [7]. At the same time, there are also new discoveries in exploring the mechanism of the influence of the lung cancer microenvironment on DCs function, which is helpful to solve the problem of inhibition of DCs function [8]. We tested the proportion of DCs in lung cancer tissues to verify the correlation between DCs and the efficacy of immunotherapy.

Methods

Flow cytometry antibodies and other reagents

Fluorescent antibody cluster of differentiation (CD)3, CD19, CD11c, purchased from BD in the United States: CD45, Human leukocyte antigen (HLA)-DR were purchased from Biolegend: LIVE/DEAD Fixable Blue Dead Cell Stain (L/D) and BV stain buffer were purchased from Sigma-Aldrich; Fetal bovine serum (FBS, 500 mL, 319801-5) was purchased from ZETA LIFE: Collagenase type IV (50 U/mL, 17104-019) was purchased from Thermo Fisher; DNase I, 20 U/mL, 10104159002 was purchased from Roche.

Sample collection

Thirty-two lung cancer samples were taken from West China Hospital of Sichuan University, Of these, 11 were in the TN group, 8 in the MPR group and 13 in the non-MPR, and the pathological diagnosis of lung cancer patients was NSCLC, see (Table 1) for clinical information. All subjects signed the informed consent form. Surgically excised human NSCLC tissue, the sample was removed from the necrotic tissue, the blood and other impurities were flushed out in PBS, placed in 10% FBS+RPMI1640, and shipped back at 4°C. The whole process is completed within 2 hours.

Variable	TN (n=11)	MPR (n=8)	non-MPR (n=13)
Sex			
Female	4	1	2
Male	7	7	11
Average age(Mean±SD)	65.92±16.2	56.27±11.73	56.4±12.69
Smoking history			
Never	5	1	4
Ever	6	7	9
Histology			
Squamous	6	5	10
Non squamous	5	3	3
Clinical staging			
IV	0	1	1
III	9	7	10
I-II	1	0	1
unknown	1	0	1

Table 1: Clinical information for patients.

Sample processing

After the tissues were retrieved, 2 mL of 10% FBS + RPMI1640 was prepared in a 10 cm Petri dish on ice, the tissues were placed in the medium, the tissue blocks were fan cut into 4 parts in the center, each part was about 3 mm×3 mm×3 mm, and the grouping started to be processed and sheared in the process in 2 h, and the corresponding digestive enzymes were added into each group respectively, and the digestion was rotated in an incubator at 37 °C for 30 min. After completion of digestion, centrifuge at 100 g for 1 min at 4 °C, remove the undigested tissue to the bottom of the tube, and aspirate the supernatant. After digestion, add 1 times the volume of 2% FBS + RPMI1640 to terminate the digestion, centrifuge at 300 g for 7 min at 4 °C, remove the supernatant. 3 mL of 2% FBS + RPMI1640 were washed, centrifuged at 300 g for 7 min at 4 °C, and the supernatant was removed. 1mL of 0.04% BSA was resuspended the precipitate, filtered through a 70 µm screen, and the original tube was washed and the residual cells were sieved through a sieve with 1mL of 0.04% BSA. Sieve. The cell suspension of each group of cells was centrifuged at 300 g for 5 min at 4 °C and resuspended with an appropriate amount of 0.04% BSA. After the cell suspension was counted, the cell suspension was adjusted to $1.0×10^7$ cells/mL and set aside.

Flow cytometry assay

A total of 32 human NSCLC tissue samples were detected and analyzed according to the established flow-through protocol. Twenty fluorescent antibodies and L/D dye were mixed in 50 μL PBS according to the titrated concentration, and 50 μL cell suspension was added and mixed thoroughly, incubated for 15 min at room temperature and protected from light, 1 mL PBS was added, centrifuged at 400 g for 5 min, washed, and the supernatant was discarded, resuspended in 300 μL PBS, and detected on the machine (Symphony A5, USA, BD Bioscience).

Data analysis

At least 106 living Events were collected and Flowjo 10.10.0 software was used for flow data analysis. Graphpad 8.0 statistical software was used for data processing and analysis. Measurements were expressed as x±s. Comparisons between groups were made using one-way ANOVA. Differences were considered statistically significant at $P<0.05$.
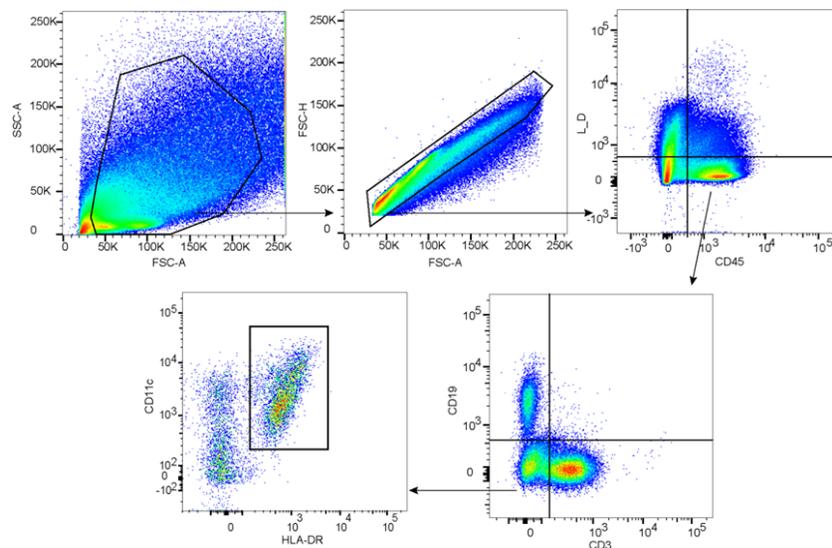
Results

Figure 1: Flow analysis and gating logic setting.

The MPR group had the lowest proportion of DCs

Through flow data analysis, it was found that the proportion of DC cells in MPR group was lower than TN group and non-MPR group, non-MPR group was higher than TN group, MPR group was the lowest and the highest in non-MPR group, as shown in (Figure 2).
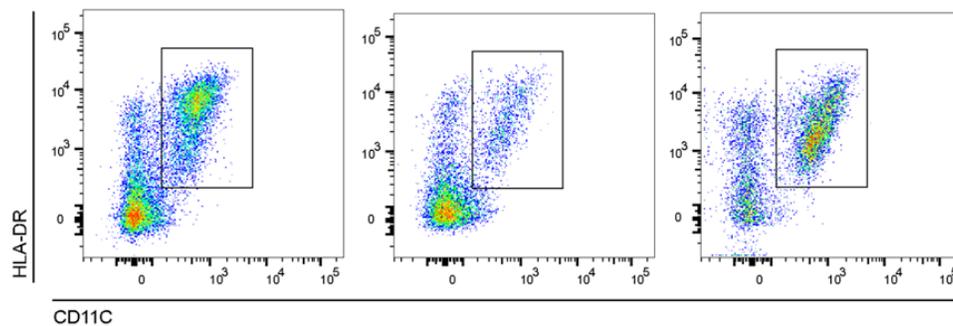

Figure 2: The portion of DCs in three groups of samples in flow scatter plot. (A). Proportion of the DCs in the TN group. (B).MPR The proportion of the group DCs. (C). Proportion of the DCs in the non-MPR group.

DCs were decreased in the MPR group and increased in the non-MPR group

Statistical analysis of the data showed that each group was TN (11), MPR (8) and non-MPR (13). MPR group had the lowest proportion in the three groups, significantly lower than non-MPR group ($p < 0.05$) and lower than TN group (no significant difference), and no significant difference between non-MPR group higher than TN group). (Figure 3).
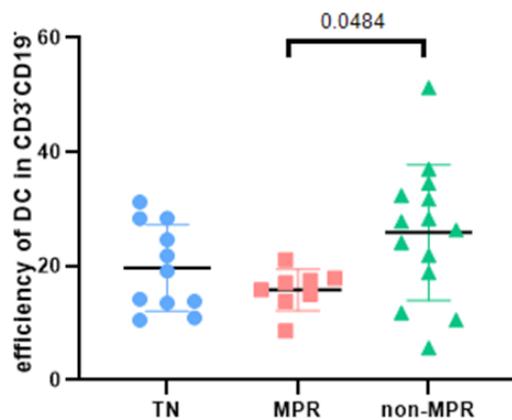

Figure 3: Statistical analysis of the proportion of DCs in the three groups.

The MPR group contributed the least in the DCs

One sample from each of the three groups was selected for tSNE dimension reduction analysis by Flowjo software to visually show the contribution of dendritic cells, with 5000 CD45 + cells in each sample after downsanple. Through the CD11c + HLA-DR + group gating to show the contribution of DCs in it. After the merge, the DC cells were clustered in the lower left corner, and the group was obvious in (Figure 3A). After reducing the contribution of the three samples, the MPR group contributed the least, the TN and non-MPR group were larger, but the non-MPR group contributed the most (Figure 4B-D).
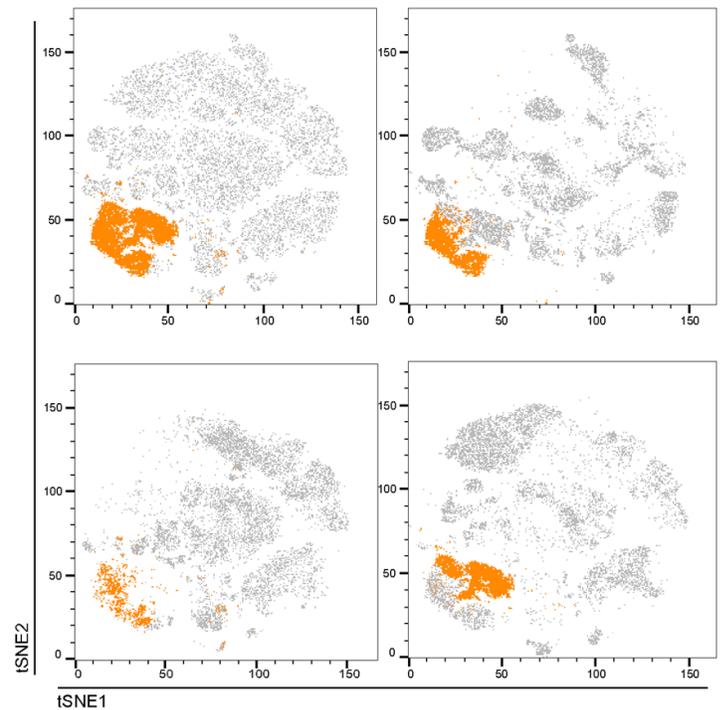

Figure 4: tSNE analyzed the DCs contributions in the three sets of samples. (A). tsne analysis of the three groups merged. (B). the contribution of DC cell subpopulations in the TN group. (C). the contribution of DC cell subpopulations in the MPR group. (D). the contribution of DC cell subpopulations in the non-MPR group.

The distribution of DCs in MPR group was less than 21.9%

The proportion of DCs in each sample was counted, and the linear distribution map of DCs in each sample was drawn by R4.4.2. The results found that the proportion of DCs in MPR group was less than 21.9%, and even some were less than 10%. However, the proportion of DCs in TN group and non-MPR group was almost higher than 10%, most of TN group were higher than 21.9%, and half of non-MPR group were higher than 21.9%, indicating that the proportion of DCs in MPR group decreased after immunotherapy, while non-MPR group did not change significantly (Figure 5).
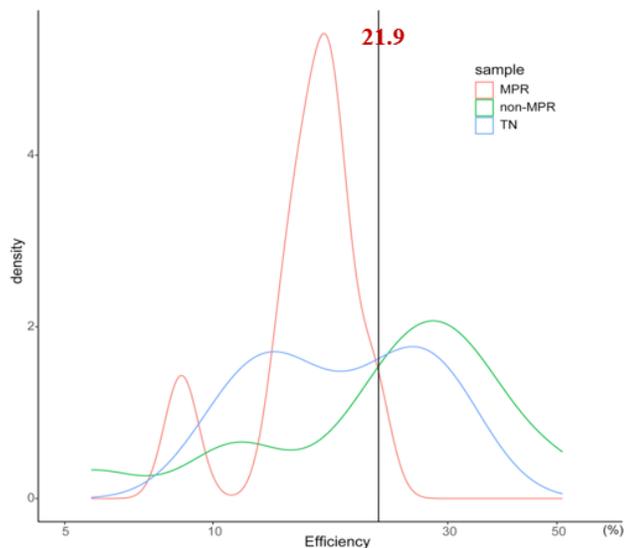
Figure 5: ggplot to analyze the distribution of DCs proportion.

Discussion

Lung cancer is known as the world's number one cancer killer, and according to the latest research data, lung cancer has the 2nd highest incidence of malignant tumors and the 1st highest mortality rate [9]. Surgical resection is the main treatment option for patients with early and some locally advanced lung cancer; however, more than 50% of patients who undergo surgery alone develop local or distant recurrence [10]. Neoadjuvant chemotherapy provides an absolute improvement of about 5% in 5-year overall survival (OS) for lung cancer patients [11-13], but also induces considerable drug toxicity. Therefore, there is an urgent need to implement new systemic therapeutic strategies for operable lung cancer patients to improve patient survival. Although neoadjuvant immune checkpoint inhibitors (ICIs) have improved the clinical benefit of some lung cancer patients [14], there is a lack of accurate biomarkers for screening the beneficiary population.

DCs have been found to be central regulators of adaptive immune responses and are therefore essential for T cell-mediated immunity to cancer [15]. DCs are located at the front line of the immune system where they actively capture antigens, such as viruses, bacteria, and mutated cancer cell fragments and then present them to T cells, usually resulting in the activation of CD8 + T cells, which eliminate infected or abnormally mutated cells [16]. Thus, dendritic cells play a vital and important role in coordinating the immune system against pathogens and cancer cells. DCs are considered to be a central component of the tumor microenvironment (TME) that promotes anti-tumor T-cell responses [17]. However, an immunosuppressed TME can affect the function of DC effectors, alter DC phenotypes, and promote dysfunction and tolerance. These outcomes are mediated through different mechanisms, including soluble mediators as well as cell-to-cell contacts [18-20]. Despite positive clinical responses to ICIs, complete clinical responses have only been observed in a small proportion of patients [21]. DCs have been shown to play a key role in the therapeutic response to ICIs and represent an effective target for cancer immunotherapy.

In this study, we used flow cytometry to resolve the molecular phenotypes of DCs associated with neoadjuvant immune-combination chemotherapy response in lung cancer patients, and found that DCs are likely to be tumor-specific cells responding to the treatment of ICIs; and combined with different dimensions of clinical indicators, we explored the potential neoadjuvant immune-combination chemotherapy response-associated biomarkers, which may provide a new screening of the population that will benefit from neoadjuvant immunotherapy for clinical lung cancer. However, the upregulation of DC cells in the non-MPR group may be related to immunosuppressive factors and cells in the different tumor microenvironment after neoadjuvant immunotherapy for lung cancer, which needs further validation [22, 23].

Author contributions

Tingting Guo formulated the hypotheses, designed the studies, carried out the experiments , provided supervision, analyzed data, and wrote the manuscript.

Declaration of interest

There has no conflict of interest.

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