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Effects of Chemotherapy on Peripheral Blood NK Cell Receptor NKG2D and Related Immune Cytokines in Patients with Non-Small Cell Lung Cancer

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Abstract

Objective: To analyze the effect of chemotherapy on peripheral blood NK cell receptor NKG2D and related immune cytokines (IL-12, IL-15, IL-18) in patients with non-small cell lung cancer (NSCLC). **Methods:** A total of 48 patients with NSCLC who visited the Oncology Department of the Affiliated Hospital of Chengde Medical College from September 2018 to September 2019 were selected as the study subjects. Changes in the expression levels of NKG2D, IL-12, IL-15 and IL-18 in peripheral blood of patients at different time points (before chemotherapy, after the first chemotherapy and after the second chemotherapy) were analyzed to investigate the correlation between NKG2D and IL-12, IL-15 and IL-18 in peripheral blood at each time point. **Results:** The expression levels of NKG2D, IL-15, and IL-18 in the peripheral blood of the patient before chemotherapy, after the first chemotherapy, and after the second chemotherapy gradually decreased. After the first chemotherapy and the second chemotherapy, the peripheral blood IL-12 was significantly lower than before chemotherapy, and IL-12 in peripheral blood after the second chemotherapy was slightly increased compared with that after the first chemotherapy. The comparison of each factor at different time points was statistically significant (all $P<0.05$). Pearson correlation analysis showed that after the first chemotherapy, NKG2D in peripheral blood was positively correlated with IL-18 ($r = 0.342$, $P = 0.031$); after the second chemotherapy, NKG2D in peripheral blood was positively correlated with IL-18 ($r = 0.411$, $P = 0.023$), negatively correlated with IL-15 ($r = -0.451$, $P = 0.001$). **Conclusion:** There was no significant change in the number of NK cells in the peripheral

blood of NSCLC patients after chemotherapy, while NKG2D and related immune cytokines decreased, which may be one of the mechanisms for the suppression of immune function in patients, and this provides a potential target for immunotherapy in patients.

Keywords

Non-Small Cell Lung Cancer, Chemotherapy, NKG2D, Immune Cytokines

1. Introduction

Lung cancer is one of the most common malignant tumors, according to incomplete statistics more than 1.3 million new cases annually worldwide, about 400,000 patients are diagnosed annually in China, with an incidence rate of 61.4/100,000 [1]. Among them, non-small cell lung cancer (NSCLC) accounts for more than 80% of the cases and is the most common subtype of lung cancer. With its invasive clinical symptoms and lack of specific early screening means, about 70% of NSCLC patients are already in advanced stages when they are diagnosed, missing the best time for treatment [2]. Currently, chemotherapy is the first-line treatment for advanced NSCLC, and it has extended the survival time of patients to a certain extent, but the overall effect is still unsatisfactory, which may be related to the toxic side effects caused by chemotherapy. Therefore, clarifying the effects of chemotherapy on the organism and giving effective interventions are crucial to improving patient prognosis. It has been suggested that chemotherapy can stimulate the release of immunosuppressive factors and thus reduce immune damage in tumours, and that chemotherapy combined with immunotherapy may enhance the anti-tumour effect, but the exact mechanism of the effect is still unclear [3]. Abnormal expression of these factors can affect the killing effect of NK cells [4] [5], and the suppression of the immune system by chemotherapy may be related to this, but little has been reported at this stage. In view of this, this study analyses the effect of chemotherapy on the peripheral blood NK cell receptor NKG2D and related immune cytokines (IL-12, IL-15, IL-18) in patients with NSCLC, with a view to providing assistance in the application of immunotherapy to patients.

2. Subjects

One hundred and two patients with NSCLC who attended the oncology department of the Affiliated Hospital of Chengde Medical College from September 2018 to March 2021 were selected as study subjects. There were 57 male cases and 45 female cases. The ages ranged from 35 to 77 years old, with a mean age of (60.278.15) years. There were 22 cases of squamous lung cancer, 71 cases of adenocarcinoma of the lung and 9 cases of adenosquamous lung cancer. TNM stage: 30 cases of stage III and 72 cases of stage IV. Inclusion criteria: 1) first

presentation and histopathologically confirmed NSCLC with TNM staging based on AJCC staging criteria (8th edition) [6]; 2) 18 - 80 years old; 3) no indication for surgery as assessed by pathology and imaging; 4) KPS score ≥ 60; 5) expected survival time of more than 3 months. Exclusion criteria: 1) immune system diseases such as rheumatism and rheumatoid diseases; 2) serious dysfunction of vital organs such as heart, lung, liver and kidney; 3) serious infectious diseases; (4) haematological system diseases. The study was approved and agreed by the Ethics Committee of the Affiliated Hospital of Chengde Medical College, while all patients signed an informed consent form.

3. Methods

General clinical data Basic clinical data of the enrolled patients were collected, including age, gender, pathological type and TNM stage (See Table 1 for details).

Chemotherapy regimen All patients in the group had no contraindications to chemotherapy and were administered cisplatin/carboplatin + pemetrexed/ gemcitabine/paclitaxel at the following doses: gemcitabine 1250 mg/m^2, pemetrexed 500 mg/m^2, paclitaxel 175 - 200 mg/m^2, cisplatin 75 mg/m^2, carboplatin AUC = 6. All doses were administered intravenously in 21-day cycles. All administered intravenously over 21 days.

Detection of biochemical parameters 10 ml of fasting venous blood was taken from patients before chemotherapy (T1), on day 21 after the first chemotherapy (T2) and on day 21 after the second chemotherapy (T3), respectively, and left to stand for 2 h at room temperature, then centrifuged at 1000 rpm for 20 min (14 cm radius), and the supernatant was kept in a refrigerator at −80˚C. The serum levels of NKG2D, IL-12, IL-15 and IL-18 were measured by ELISA, and the kits for each factor were purchased from Kangtai Heyuan Biotechnology Co.

Statistical treatment Data analysis for this study was performed using SPSS version 23.0 (SS Inc, Chicago, IL). Measures conforming to a normal distribution were expressed using the mean standard deviation, trends in peripheral blood factors over time were tested using ANOVA with one-way repeated measures data, and comparisons of levels of each factor at different time points were performed using the Bonferroni method. Correlations between NKG2D and IL-12, IL-15 and IL-18 at each time point were analysed using Pearson

Table 1. General clinical date.

Age	Gender (male/ female)	athological type (squamous/adenocarcinoma/ adenosquamous carcinoma)	Staging (III/IV)	Chemotherapy regimen (doxorubicin + isplatin/gemini + cisplatin/pemet + carboplatin/pemet + cisplatin/paclitaxel + carboplatin/purple shirt + carboplatin)
60.27 ± 8.15	57/45	22/71/9	30/72	27/16/32/24/1/2

correlation. All P values were tested two-sided and $P < 0.05$ was considered a statistically significant difference.

4. Results

Changes in the number of NK cells, there was no statistically significant change in the number of NKs in each patient after the 1st chemotherapy and after the 2nd chemotherapy compared to the pre-chemotherapy period (See Table 2 for details).

Changes in the levels of NKG2D and IL-12, IL-15 and IL-18 in peripheral blood over time The expression levels of NKG2D, IL-15 and IL-18 in peripheral blood decreased gradually before, after the 1st chemotherapy and after the 2nd chemotherapy in patients, and IL-12 in peripheral blood decreased significantly after the 1st chemotherapy and after the 2nd chemotherapy in patients compared with the pre-chemotherapy period, while after the 2nd chemotherapy IL-12 in peripheral blood increased slightly compared to post-chemotherapy 1. There was a significant difference between the two comparisons at different time points for each factor (*$p < 0.05$, the different was statistically significant) (See Table 3 and Figure 1 for details).

Correlation of NKG2D with IL-15, IL-12 and IL-18 at different time points Pearson correlation analysis showed that: before chemotherapy, NKG2D in peripheral blood did not correlate with IL-18, IL-12 and IL-15; after the patient's 1st chemotherapy, NKG2D in peripheral blood correlated positively with IL-18 (r =

Table 2. Changes of NK cell numbers.

Subgroups	Pre-chemotherapy	Post-chemotherapy 1 cycle	Post-chemotherapy 2 cycles	Statistical values	p
NK cell count	2.261 ± 0.2471	2.308 ± 0.2503	2.342 ± 0.2417	F = 1.095	0.338

Table 3. Changes of NKG2D and IL-12, IL-15 and IL-18 levels in peripheral blood over time.

Parameter	Number of cases	NKG2D (ng/ml)	IL-18 (pg/ml)	IL-12 (pg/ml)	IL-15 (pg/ml)
T1	102	2.12 ± 0.16	134.17 ± 11.29	12.07 ± 0.26	5.10 ± 0.09
T2	102	1.85 ± 0.09	94.08 ± 5.96	7.42 ± 2.18	4.19 ± 1.01
T3	102	1.32 ± 0.24	54.38 ± 9.84	8.08 ± 0.98	2.87 ± 0.09
F-value	-	125.134	1277.012	144.981	71.215
P-value	-	<0.001*	<0.001*	<0.001*	<0.001*
Intra-group comparison (P)					
T1 vs T2	-	<0.001	<0.001	<0.001	<0.001
T1 vs T3	-	<0.001	<0.001	<0.001	<0.001
T2 vs T3	-	<0.001	<0.001	0.006	<0.001

0.342, P = 0.031) and no correlation with IL-12 and IL-15; NKG2D in peripheral blood after the patient's 2nd chemotherapy showed a positive correlation with IL-18 (r = 0.411, P = 0.023), a negative correlation with IL-15 (r = −0.451, P = 0.001, P < 0.05, the different was statistically significant) and no correlation with IL-12 (See Table 4 and Figure 2 for details).

5. Discussions

In the body's anti-tumour immune process, NK cells are the most important

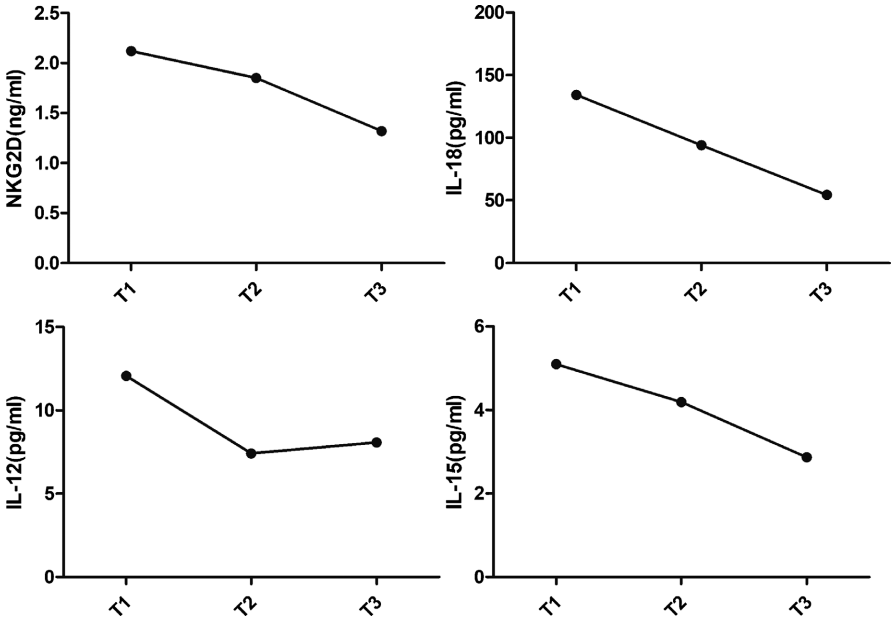

Figure 1. Changes of NKG2D and IL-12, IL-15 and IL-18 levels in peripheral blood over time.

Table 4. Correlation between NKG2D and IL-12, IL-15 and IL-18 at different time points.

Parameter	NKG2D (T1)		NKG2D (T2)		NKG2D (T3)	
	r	*P*	*r*	*P*	*r*	*P*
IL-18	0.09	0.585	0.342	0.031	0.411	0.023
IL-12	0.08	0.641	−0.068	0.709	−0.239	0.091
IL-15	0.006	0.898	0.003	0.897	−0.451	0.001*

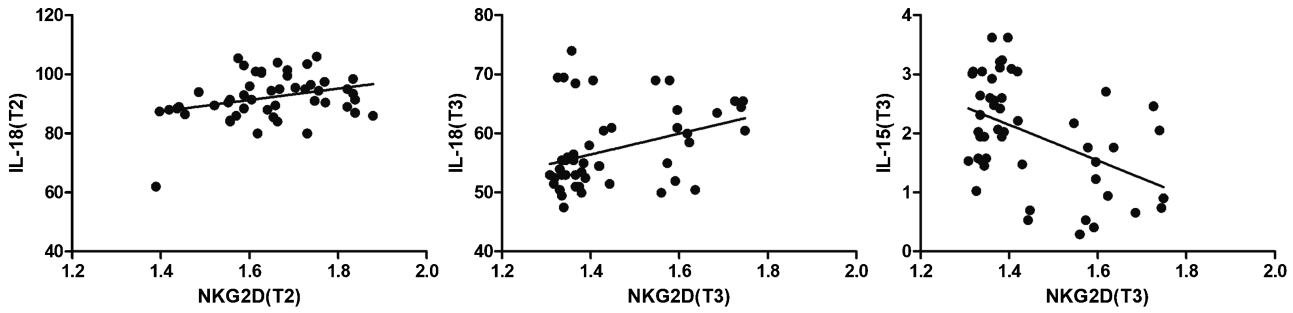

Figure 2. Correlation between NKG2D and IL-12, IL-15 and IL-18 at each time point.

class of intrinsic immune effector cells, which can activate relevant ligands through the tumour-deficient major histocompatibility antigen-I (MHC-I) class of molecules, thereby promoting the secretion of immune effectors and cytokines, and exerting their immune killing effect on tumours [7]. Chemotherapy is one of the effective treatments for malignant tumours, but it is long and non-specific, killing tumour cells while damaging normal tissues to varying degrees, the most important of which is the phased removal of lymphocytes, thereby suppressing normal immune function [8]. The first-line treatment for advanced NSCLC is platinum-based combination chemotherapy, but the overall survival time of patients is only about 10 months, and the 2-year survival rate is only about 10% [9]. The reasons for the poor prognosis of patients include tumour invasion and the suppression of the body's immune system by chemotherapy.

NKG2D is a member of the NKG2 family and is the main active receptor mediating the killing activity of NK cells and is the target of immune editing between NK cells and tumour cells. The normal expression of NKG2D is the basis for the killing effect of NK cells and is one of the decisive factors in maintaining the immune function of the body [10]. Nie [11] demonstrated that serum NKG2D levels were significantly reduced in lung cancer patients, and the suppression of cellular immune function was obvious and closely related to tumor progression. A study by Yuan Lifang [12] found that the level of NKG2A, an inhibitory receptor on the surface of NK cells, was significantly increased and the NKG2A/NKG2D ratio was significantly decreased in the peripheral blood of lung cancer patients, suggesting that one of the mechanisms of immune escape of tumor cells may be related to the imbalance of NKG2A and NKG2D expression of NK cell surface receptors. All these studies illustrate that the reduced expression of NKG2D seriously affects the immune function of the body. The results of this study showed that the levels of peripheral blood NKG2D in NSCLC patients were significantly lower after chemotherapy compared to those before chemotherapy, and gradually decreased with the extension of chemotherapy cycles, suggesting that the decrease in NKG2D levels caused by chemotherapy in patients may be one of the reasons for the impairment of immune function. The subjects enrolled in this study were all patients with advanced NSCLC without indications for surgery. The long-term application of chemotherapeutic drugs led to the systematic removal of normal immune cells, and the destruction of NK cells was particularly severe, which in turn caused a decrease in the level of the surface active receptor NKG2D, as well as a depletion of NKG2D to enhance the killing effect of NK cells. In addition, the higher aggressiveness of advanced NSCLC, which causes elevated expression of the inhibitory receptor NKG2A, also inhibits the expression of NKG2D to some extent.

IL-12 is a cytokine secreted by monocytes, dendritic cells and other antigen-presenting cells, and belongs to the class I cytokine receptor family. IL-15 is mainly secreted by macrophages and its effect is similar to that of IL-12, mainly promoting the activation and proliferation of NK cells, as well as the secretion of

INF- and TNF-α to enhance the cellular immunity of human cells [13] [14] [15]. The cytotoxicity of spleen lymphocytes was also significantly increased. In a study by Zhao Jiaxiu [16], IL-12 was found to promote the expression of NKG2D receptors on the surface of NK cells. In a study by Mei Jia-Tuan *et al.* [17], IL-15 was shown to increase the expression of NKG2D on the surface of NK cells, thereby enhancing their tumour-killing effects. IL-18 is also an immunostimulatory cytokine that enhances the NK cell-mediated anti-tumour immune response and promotes the secretion of INF- and TNF-α, which play an important role in the human anti-tumour immune mechanism [18]. Qi Yuanying's study [19] found that IL-12 combined with IL-18 increased the killing activity of NK cells against tumour cells and this synergistic induction was achieved by promoting the expression of NKG2D. The results of this study showed that IL-12, IL-15 and IL-18 in peripheral blood decreased significantly after chemotherapy in NSCLC patients and gradually decreased with the extension of chemotherapy application time, suggesting that chemotherapy inhibits the expression of NK cell activating factor, which is also one of the mechanisms of immunosuppression. The massive application of chemotherapy in patients with advanced NSCLC leads to damage to normal tissues, causing a decrease in the secretion of IL-12, IL-15 and IL-18, which in turn leads to impaired expression of NKG2D and a weakening of the killing effect of NK cells, and at the same time, the depletion of such cytokines for the activation of NK cells also leads to a further decrease in their levels.

In summary, there was no significant change in the number of NK cells in the peripheral blood of NSCLC patients after chemotherapy, while NKG2D and related immune cytokines decreased, which may be one of the mechanisms for the suppression of immune function in patients, and this provides a potential target for immunotherapy in patients. The shortcomings of this study are that the patients enrolled in the study were from a single centre and the chemotherapy cycle was still short, which may cause bias in the results, and the sample size should be expanded and the study period extended in the future to confirm this conclusion.

6. Conclusion

In this study, we analyzed the correlation between NKG2D and the crowd factors at each time point, and the results showed that NKG2D only had a weak correlation with IL-15 and IL-18. This result seems to be contrary to IL-12, IL-15 and IL-18 promoting the expression of NKG2D, but in fact, it is not. There are many factors affecting the immune function of patients with advanced NSCLC, besides the damage of chemotherapeutic drugs, it is also related to the invasive tumor damage, and the immune reconstruction of the body during chemotherapy also restores the related cells and factors [20], thus leading to the poor correlation between NKG2D and its related factors.

Fundamental Project

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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