

RESEARCH ARTICLE

CD3⁺ CD4⁺ and CD3⁺ CD8⁺ Lymphocyte Subgroups and their Surface Receptors NKG2D and NKG2A in Patients with Non-small Cell Lung Cancer

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Abstract

Background: To explore the prevalence of lymphocyte subgroups CD3⁺ CD4⁺ and CD3⁺ CD8⁺ and their surface receptors NKG2D and NKG2A in patients with non-small cell lung cancer (NSCLC). **Materials and Methods:** A total of 40 patients with NSCLC were divided into different groups according to different clinical factors (TNM staging, pathological patterns and genders) for assessment of relations with CD3⁺ CD4⁺ and CD3⁺ CD8⁺ and the surface receptors NKG2D and NKG2A of T lymphocytes in peripheral blood by flow cytometry. **Results:** Patients in the advanced group had evidently lower levels of CD3⁺ CD4⁺ but markedly higher levels of CD3⁺ CD8⁺ in peripheral blood than those with early lesions ($p < 0.05$). In addition, NSCLC patients in the advanced group had obviously higher CD3⁺ CD4⁺ NKG2D and CD3⁺ CD8⁺ NKG2A expression rates but lower CD3⁺ CD4⁺ NKG2A and CD3⁺ CD8⁺ NKG2D expression rates ($p < 0.05$). However, there were no significant differences between NSCLC patients with different genders and pathological patterns in expression levels of lymphocyte subgroups CD3⁺ CD4⁺ and CD3⁺ CD8⁺ and their surface receptors NKG2D and NKG2A. **Conclusions:** Unbalanced expression of surface receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ lymphocytes may be associated with a poor prognosis, greater malignancy and immunological evasion by advanced cancers, related to progression of lung cancer.

Keywords: Non-small cell lung cancer - lymphocyte subgroup - blood - flow cytometer

Asian Pac J Cancer Prev, 15 (6), 2685-2688

Introduction

Lung cancer is the primary risk factor of malignant tumor-associated death across the world in the 21st century with increasingly rising morbidity and mortality, which has no significant clinical characteristic and symptom as well as specific detection method in its early stage (Kim et al., 2014; Deng et al., 2014). Therefore, patients with lung cancer are always in advanced ones when diagnosed with highly limited recovery rate by systematic treatment, for which early diagnosis and treatment become one of the topics in studying lung cancer (Huang et al., 2013; Liu et al., 2014). In recent years, with the rapid development of molecular biology and immunology and the multidisciplinary cross integration, achievements have been made in the research of mutual regulation among immunological cells in tumor tissues (Sánchez et al., 2014; Liu et al., 2014). Of the examination indexes for diagnosing the development, progression and prognosis of tumors, the detection of T lymphocyte subgroups in peripheral blood is the most popular one in clinic (Xiao et al., 2013; Zhao et al., 2014). This study investigated the expressions and relevant factors of lymphocyte subgroups CD3⁺ CD4⁺ and CD3⁺ CD8⁺ and their surface receptors NKG2D and NKG2A in patients with NSCLC.

Materials and Methods

General data

A total of 40 patients diagnosed with NSCLC by pathological examinations admitted in our hospital from March 2010 to May 2012 were selected as study objects, in which there were 23 males and 17 females, aging 47~65 years, with average age being (57.3±4.3) years. TNM staging: 10 patients in phase I, 13 in phase II, 10 in phase III and 7 in phase IV. Pathological patterns: 25 patients with squamous carcinoma and 15 with adenocarcinoma. Exclusion criteria: 1 Patients who had treated with surgeries, radiotherapies and chemotherapies when examined by immune function detection; 2 Patients who had tumor history; 3 Patients who were accompanied by autoimmune disease, such as insulin-dependent diabetes, hyperthyroidism, systemic lupus erythematosus and rheumatoid arthritis, etc.; 4 Patients who had taken hormonal drugs; 5 Patients who had received any immunosuppressive agents.

Methods

5 mL fasting venous blood of each patient was collected at early morning to analyze the expressions of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ and their surface receptors

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NKG2D and NKG2A by flow cytometer after anti-coagulation. All patients were divided into different groups to analyze the detection results according to different clinical factors, such as YNM staging, pathological patterns and genders. The peripheral blood samples were divided into early group (phases I and II, 23 cases) and advanced group (phases III and IV, 17 cases) according to TNM staging; squamous carcinoma group (25 cases) and adeno-carcinoma group (15 cases) bases on pathological patterns; and male group (23 cases) and female group (17 cases) on the basis of different genders.

Statistical data analysis

SPSS17.0 software was applied to conduct t test ($\alpha=0.05$), variance analysis and correlation analysis. $p<0.05$ was considered to be statistically different.

Results

Expression comparisons of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients with different TNM staging

NSCLC patients in advanced group was evidently lower in expression of CD3⁺ CD4⁺ but markedly higher in expression of CD3⁺ CD8⁺ in peripheral blood than early group, and the differences were significant ($p<0.05$) (Table 1).

Expression comparisons of surface receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients with different TNM staging

NSCLC patients in advanced group was obviously higher in CD3⁺ CD4⁺ NKG2D and CD3⁺ CD8⁺ NKG2A expression rates but apparently lower in CD3⁺ CD4⁺ NKG2A and CD3⁺ CD8⁺ NKG2D expression rates than early group, and there were significant differences ($p<0.05$) (Table 2).

Expression comparisons of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients with different pathological patterns

There were no significant differences between squamous carcinoma group and adeno-carcinoma group in expressions of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients ($p>0.05$) (Table 3).

Expression comparisons of surface receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients with different pathological patterns

The differences in the expressions of surface receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients between squamous carcinoma group and adeno-carcinoma group were not significant ($p>0.05$) (Table 4).

Expression comparisons of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients with different genders

There were no significant differences between male group and female group in expressions of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients ($p>0.05$) (Table 5).

Expression comparisons of surface receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients with different genders

The differences in the expressions of surface receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in peripheral blood of NSCLC patients between male group and female group were not significant ($p>0.05$) (Table 6).

Discussion

In the immunological defense mechanism of body to tumors, cellular immunity plays an predominant function,

Table 1. Expression Comparisons of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in Peripheral Blood of NSCLC Patients with Different TNM Staging (% , $\bar{x}\pm s$)

Groups	n	CD3 ⁺ CD4 ⁺	CD3 ⁺ CD8 ⁺
Early group	23	39.66±5.41	22.56±2.14
Advanced group	17	30.22±4.36*	33.65±3.47*

*Compared with early group, * $p<0.05$.

Table 3. Expression Comparisons of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in Peripheral Blood of NSCLC Patients with Different Pathological Patterns (% , $\bar{x}\pm s$)

Groups	n	CD3 ⁺ CD4 ⁺	CD3 ⁺ CD8 ⁺
Squamous carcinoma group	25	37.36±3.41	25.12±2.04
Adeno-carcinoma group	15	36.22±3.16	26.15±2.07

Table 2. Expression Comparisons of Surface Receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in Peripheral Blood of NSCLC Patients with Different TNM Staging (% , $\bar{x}\pm s$)

Groups	n	CD3 ⁺ CD4 ⁺ NKG2D	CD3 ⁺ CD4 ⁺ NKG2A	CD3 ⁺ CD8 ⁺ NKG2D	CD3 ⁺ CD8 ⁺ NKG2A
Early group	23	2.36±0.56	5.23±0.78	95.56±2.22	7.12±0.45
Advanced group	17	6.56±0.61*	2.31±0.41*	90.12±3.21*	12.45±1.30*

*Compared with early group, * $p<0.05$

Table 4. Expression Comparisons of Surface Receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in Peripheral Blood of NSCLC Patients with Different Pathological Patterns (% , $\bar{x}\pm s$)

Groups	n	CD3 ⁺ CD4 ⁺ NKG2D	CD3 ⁺ CD4 ⁺ NKG2A	CD3 ⁺ CD8 ⁺ NKG2D	CD3 ⁺ CD8 ⁺ NKG2A
Squamous carcinoma group	25	4.66±0.36	3.23±0.38	91.56±4.22	10.52±0.45
Adeno-carcinoma group	15	4.26±0.31	3.81±0.21	92.12±2.25	11.32±1.50

Table 6. Expression Comparisons of Surface Receptors NKG2D and NKG2A in CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in Peripheral Blood of NSCLC Patients with Different Genders(%, $\bar{x} \pm s$)

Groups	n	CD3 ⁺ CD4 ⁺ NKG2D	CD3 ⁺ CD4 ⁺ NKG2A	CD3 ⁺ CD8 ⁺ NKG2D	CD3 ⁺ CD8 ⁺ NKG2A
Male group	23	4.42±1.36	2.93±0.58	93.45±9.07	9.69±1.35
Female group	17	5.06±2.31	3.01±0.91	92.66±8.25	10.32±2.50

Table 5. Expression Comparisons of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in Peripheral Blood of NSCLC Patients with Different Genders (%, $\bar{x} \pm s$)

Groups	n	CD3 ⁺ CD4 ⁺	CD3 ⁺ CD8 ⁺
Male group	23	35.31±3.11	25.01±2.14
Female group	17	36.08±3.20	26.02±2.17

whereas immunological system has rejecting activity on tumors induced by virus as well as physical and chemical carcinogens, in which T lymphocyte mediated cellular immunity exerts a critical function (Mamlouk et al., 2014; Xia et al., 2013; Wang et al., 2013). As a complex and inhomogenous community, mature T lymphocytes can be divided into two subgroups CD3⁺ CD4⁺ and CD3⁺ CD8⁺ according to different surface markers and differentiation antigens in CD molecules.

Helper T lymphocyte (Th) shares the same phenotype with CD4⁺ T cells, which is an important immune-regulation cell in human body and can assist the differentiation of B cells into antibody-secreting cells and improve the functional activation of other T cells. In addition, it is also called supplementary/induced T lymphocyte due to its functions of improving immunological responses and induce the activation of other T lymphocyte subgroups, and has positive regulatory effect on cellular immunity in that it can secrete cytokines to strengthen the anti-inflammatory mechanism of phagocytes and directly induce cellular apoptosis. CD3⁺ CD8⁺ is crucial in regulating multiple immunological mechanisms, and the primary effect cell in the responses of virus and tumor immunology as well as the transplant rejection reaction, which has negative regulatory activity in cellular immune responses. Researches discovered that the stability grade of internal environment in immunological system was in connection with the equilibrium degree among T lymphocyte subgroups, which meant that the broken equilibrium of T lymphocyte subgroups could induce immune dysfunction in human body (Remark et al., 2013; Iyengar et al., 2013). Moreover, it was also found that the immunological system in cancer patients was constantly in immunosuppressive condition, which was in potential relationship with the development and progression of tumors.

CD3⁺ CD4⁺ T lymphocytes can activate monocytes, macrophagocytes and NK cells by self-secreted cytokines, thus function as tumor killer through immune response induced by monocytes, macrophagocytes and NK cells. Additionally, they can also secrete IL-2 as the second signal to activate CD3⁺ CD8⁺ T lymphocytes, and then involve in the tumor immune responses (Xia et al., 2013), indicating that CD3⁺ CD4⁺ T lymphocytes are critical in tumor immunity in human body.

T lymphocytes-mediated immune responses play

a leading role in the anti-tumor immunity, and the expressions of T cell subgroups in peripheral blood are the better indexes and parameters to reflect the T lymphocyte immunological function status. A study demonstrated that there were abnormal T lymphocyte subgroups in peripheral blood, marked by decreased CD3⁺ CD4⁺ expression and increased CD3⁺ CD8⁺ expression (Kuhn et al., 2013). Another study reported that the immunological function in patients with lung cancer was often in immunosuppressive condition, which was caused by multiple immunosuppressive factors (Ganesan et al., 2013). These factors could inhibit the maturity and differentiation of CD3⁺ CD4⁺ cells, which reduced the amounts of CD3⁺ CD4⁺ cells and weaken the immunological surveillance of body on tumor cells. Meanwhile, the amount of CD3⁺ CD8⁺ cells increased evidently, bringing about reduced functions in recognizing and killing tumor cells.

In this study, NSCLC patients in advanced group was evidently lower in expression of CD3⁺ CD4⁺ but markedly higher in expression of CD3⁺ CD8⁺ in peripheral blood than early group, certified that the cellular immunity of patients with malignant tumor was inhibited and was in close correlation with the TNM staging of lung cancer, demonstrating that the suppressive severity of cellular immunological function was more significant in patients with advanced lung cancers, becoming one of the potential reasons for sever disease condition and poor prognosis, whose detailed mechanism needs to be further studied. However, there were no significant differences in the expressions of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ in NSCLC patients with different pathological patterns and genders, and it needs to be further explored whether there is any influence on immunological functions.

NKG2D and NKG2A are the most effective receptors in the regulation of cellular immunological reactions and can selectively express on the molecular surfaces of NK cells and some T lymphocytes (Liu et al., 2013). In immunological response, they combine with specific ligands first, and then transduce chemical signals into cells, which can regulate the cellular immunity mediated by NK cells and T lymphocytes, impacting the killing activity of immunological cells on tumors (park et al., 2011). CD3⁺ CD4⁺ NKG2D and CD3⁺ CD8⁺ NKG2D cells proliferate and produce FasL under the synchronizing stimulant of NKG2D and MIC, while FasL has reverse regulatory action on the proliferation of T lymphocytes under the condition lacking of NKG2D, showing evidence that NKG2D ligand has certain influence on CD3⁺ CD4⁺ and CD3⁺ CD8⁺ cells, which gave rise to the unbalanced expressions of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ T lymphocytes and inhibited immunological functions of tumors in human body. With the increase of malignant severity of tumors, partial membrane-bound ligand could be hydrolyzed to

form soluble ligand, leading to the decrease of CD3⁺ CD4⁺ NKG2D expression level. In this study, NSCLC patients in advanced group was obviously higher in CD3⁺ CD4⁺ NKG2D expression rate but apparently lower in CD3⁺ CD8⁺ NKG2D expression rate than early group, which were consistent with the above reports.

NKG2A is a kind of inhibitive receptor, which can inhibit the killing function of cellular immunity on tumors by recognizing ligand molecules and their submitted specific peptide fragments expressed on the surface of tumor cells (Wrobel et al., 2007). The results of this study indicated that NSCLC patients in advanced group was obviously higher in CD3⁺ CD8⁺ NKG2A expression rate but apparently lower in CD3⁺ CD4⁺ NKG2A and expression rate than early group, according to different TNM staging, suggesting that because of the different time of TNM staging, the expression of NKG2A ligands on the surface of T lymphocytes might increase or decrease relevantly, bringing about different suppressive severity on cellular immunity, and autologous ability of tumor resistance was in negative relationship with the increase of TNM staging.

The integrated signals transmitted by relevant ligands recognized by NKG2D and NKG2A respectively serving as the activated and inhibitive receptors on the surface of T lymphocytes have certain influence on the expression levels of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ T cells. With the increase of TNM staging, the expression of subgroup CD3⁺ CD4⁺ with positive regulatory action on tumor cell immunity decreased while CD3⁺ CD8⁺ with positive regulatory action increased, causing immunological dysfunction in human body, which put the immunological system into suppressed condition. However, the expression levels of CD3⁺ CD4⁺ and CD3⁺ CD8⁺ mainly depend on the comprehensive changes of the expression levels of NKG2D and NKG2A, which is in accordance with the decreased cellular immunity in human body along with the increase of TNM staging, suggesting that the higher the malignant severity of advanced tumors and the invasive ability of cells are, the worse the prognosis becomes. Additionally, it is predicated that the expression changes of lymphocyte subgroups CD3⁺ CD4⁺ and CD3⁺ CD8⁺ and their surface receptors NKG2D and NKG2A are associated with the immunological escape of tumors, whose mechanism needs to be further studied.

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