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Original Article

Decreased immune function during aging may be the endogenous cause of lung cancer

Li Wei[1*], Yupeng Du[2*], Wei Wu[1], Jing Wang[1], Shanshan Wu[1], Qi Xia[1]

[1]Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory of Diagnostic and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China; [2]Department of Rehabilitation, The Third Hospital of Zhejiang University of Chinese Medicine, Hangzhou, China. [*]Equal contributors.

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Abstract: Objective: Our research aimed to evaluate the immune function of lung cancer patients in different statuses by analyzing T lymphocyte subsets and immunoglobulins. Methods: A total of 135 lung cancer patients were separately divided into three groups based on different factors (ages, TNM stages, and treatments). T lymphocyte subsets and natural killer (NK) cells in blood and immunoglobulins and complement in serum were analyzed in 135 lung cancer patients and 44 normal controls. Results: Our research indicated that the immune function of lung cancer patients exhibited obvious alterations during aging. Specifically, the frequency of $CD4^+$ T cells and the $CD4^+/CD8^+$ ratio significantly decreased with aging, whereas IgA, IgG, and C4 levels and the frequency of $CD8^+$ T cells significantly increased with aging. Conclusions: In our study, immune function exhibited obvious reduction trends during aging, indicating that immunosenescence during aging may represent the endogenous cause of lung cancer.

Keywords: Lung cancer, immune function, age, T lymphocyte subsets, immunoglobulins

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Lung cancer accounts for approximately 1.4 million deaths every year, and these values are increasing yearly [1, 2].

The immune system plays an important role in the battle of the host against cancer development. Although a connection between immunodeficiency and oncogenesis has been proposed, a detailed immune profile in cancer has not been well described to date [3].

Given that lung cancer is a progressive disease, it is necessary to evaluate the dynamic changes that occur in immune function based on the patient's health. T lymphocytes are recognized as the main effectors of cellular immune responses. The numbers of T lymphocyte subsets and functional changes correlate with tumor occurrence, development, and diffusion [4, 5]. Immunoglobulins also play an important role in anti-tumor and anti-infection activities and immune surveillance by promoting phagocytosis and eliminating target cells [6]. Numerous factors, such as age, TNM stage and treatment, may influence immune function in lung cancer patients and disease prognosis. However, limited information is available on detailed immune function alterations in lung cancer patients in different subgroups. Thus, studies of the dynamic changes in immune function according to different subgroups may provide references for the clinic.

Therefore, we evaluated the immune function of lung cancer patients by analyzing T lymphocyte subsets and immunoglobulins in patients based on different factors (age, TNM stage, and treatments) [7-9] to explore the immune function characteristics of lung cancer patients during disease progression.

Materials and methods

Subjects

A total of 135 lung cancer patients aged 33-81 years (mean (59.35±9.40) hospitalized at the Chest Oncology Department were recruited be-

Table 1. Frequencies of T lymphocyte subsets and NK cells in lung cancer patients and normal controls

Group	$CD3^+$ T cells (%)	$CD4^+$ T cells (%)	$CD8^+$ T cells (%)	$CD4^+/CD8^+$	NK cells (%)
Lung Cancer	72.14±10.92	39.14±11.29	29.61±12.84	1.61±0.80	15.6±9.76
Control	75.07±6.57	45.35±5.69	22.82±5.63	2.14±0.70	12.39±5.66
F	14.04	14.09	10.86	0.60	12.94
P	0.03	0.00	0.00	0.00	0.01

Table 2. Serum immunoglobulins and complement factors in lung cancer patients and normal controls

Group	IgA	IgG	IgM	C3	C4
Lung Cancer	240.67±95.15	1181.72±301.49	143.01±203.72	115.76±28.27	30.86±20.27
Control	229.52±72.30	900.82±85.67	86.32±23.42	99.05±26.67	29.36±19.65
F	3.00	29.80	2.80	0.89	0.40
P	0.48	0.00	0.07	0.00	0.67

tween January 2013 and December 2015. Diagnosis and TNM stage were performed based on clinical manifestation and laboratory examination, and the pathology was diagnosed with reference to the International Union Against Cancer (UICC) TNM stage (7th edition) standard for lung cancer. All of the patients were divided into three groups based on age (age ≤50: 17 cases, age >50 and age <70: 97 cases, age ≥70: 21 cases); three groups based on TNM stage (I: 62 cases, II:15 cases, III~IV: 58 cases); three groups based on treatment (Untreated: 14 cases, operation: 43 cases, chemotherapy or radiotherapy (chemo radiotherapy): 78 cases); and two groups based on metastasis (metastasis: 45 cases, no metastasis: 90 cases). Forty-four age- and gender-matched healthy individuals who received physical examinations in our hospital were enrolled as normal controls.

Materials and methods

Heparin anticoagulant venous blood (5 ml) was collected from each subject in a fasting state between 08:00 am and 09:00 am on d2 after admission. T lymphocyte subsets and NK cells of the blood were analyzed by flow cytometry (Beckman Coulter FC500). The antibodies for T lymphocyte subsets were purchased from BD (Per CP mouse anti-human CD3, FITC mouse anti-human CD4, PE mouse anti-human CD8). The antibodies for NK cells were purchased from Beckman Coulter Company (FITC mouse anti-human CD3, PE mouse anti-human CD16+56).

Five ml blood was collected from each subject in a fasting state between 08:00 am and 09:00 am on d2 after admission. The serum was separated by centrifugation for 10 min at 3000 r/min after clotting. In addition, immunoglobulins (IgA, IgG, and IgM) and complement factors (C3, C4) were measured by the clinical laboratory in our hospital.

Statistical analyses

Data were analyzed using SPSS 13.0 software. All data are presented as the mean ± standard deviation (SD). Descriptive statistics were used to determine whether all data were normally distributed. Comparison between two groups was performed using an independent sample t test. Comparisons among groups were determined by one-way analysis of variance (ANOVA). Differences between groups were determined using a post hoc test. $P<0.05$ was considered to be statistically significant.

Results

T lymphocyte subsets and NK cells of lung cancer patients and normal controls (compared using an independent sample t test)

Compared with *normal* controls, the frequencies of $CD3^+$ T cells, $CD4^+$ T cells, and the $CD4^+/CD8^+$ ratio were significantly decreased ($P<0.05$) in patients, whereas the frequencies of $CD8^+$ T cells and NK cells were significantly increased ($P<0.01$, **Table 1**).

Table 3. T lymphocyte subsets and NK cells in lung cancer patients from different subgroups

Group	n	$CD3^+$ T cells (%)	$CD4^+$ T cells (%)	$CD8^+$ T cells (%)	$CD4^+/CD8^+$	NK cells (%)
Age (years)						
<50	21	69.39±11.86	40.52±8.46	26.00±10.44	1.85±0.90	18.34±11.99
>50 and <70	97	71.50±10.66	38.65±10.82*	28.87±11.54**	1.58±0.75*	16.54±9.83
>70	17	73.93±11.83	32.81±10.75*	37.99±17.58**	1.16±0.79**	12.24±10.23
Treatment						
Untreated	14	72.58±9.94	39.97±8.12	29.38±10.24	1.63±0.95	15.58±8.96
Operation	43	75.34±11.11##	41.69±11.21##	31.09±13.67	1.63±0.80	12.25±8.07##
Chemoradiotherapy	78	69.63±10.54	35.65±10.35	29.55±13.03	1.48±0.76	18.14±10.97
TNM						
I	62	72.94±11.54	40.06±11.10	29.75±13.04	14.45±9.72	1.63±0.81
II	15	68.83±10.15	37.55±9.77	28.29±10.87	20.37±12.31	1.55±0.71
III~IV	58	70.91±10.54	35.87±10.28	30.53±13.47	16.75±10.03	1.46±0.80
Metastasis						
No	90	72.17±10.69	39.85±10.24	28.92±12.16	1.66±0.82	15.55±10.24
Yes	45	70.50±11.59	34.24±10.80	31.93±14.30	1.33±0.68	17.20±10.32
P		0.41	0.00	0.20	0.02	0.38

*: P<0.05 Compared with age <50; **: P<0.01 Compared with age <50; ##: P<0.01 Compared with chemoradiotherapy.

Serum immunoglobulins and complement factors in lung cancer patients and normal controls (compared using an independent sample t test)

Compared with normal controls, IgG and C3 levels were significantly decreased in patients (P<0.01, **Table 2**).

T lymphocyte subsets and NK cells of lung cancer patients with different statuses (ages, treatments, and TNM stages) were compared using one-way analysis of variance (ANOVA). Differences between groups were determined using post hoc tests. Differences between patients with or without metastasis were determined using independent sample t tests.

Obvious differences in T lymphocyte subsets and NK cells were noted among lung cancer patients from different subgroups. The frequency of $CD4^+$ T cells and the $CD4^+/CD8^+$ ratio decreased significantly as age increased (P< 0.05), whereas the frequency of $CD8^+$ T cells increased significantly as age increased (P< 0.01). Compared with the chemoradiotherapy group, the frequencies of $CD3^+$ T cells and $CD4^+$ T cells significantly increased, whereas the frequency of NK cells was reduced in the operation group (P<0.01). Compared with the metastasis group, the frequency of $CD4^+$ T cells and the $CD4^+/CD8^+$ ratio in the no metastasis group was significantly decreased (P<0.05). However, no obvious differences were noted among different TNM stage groups (**Table 3**).

Serum immunoglobulins and complement factors in lung cancer patients from different subgroups (age, treatment, and TNM stages) were compared using one-way analysis of variance (ANOVA). Differences between groups were determined using post hoc tests. Differences between patients with or without metastasis were determined using independent sample t tests.

Obvious differences in immunoglobulins and complement factors were noted among lung cancer patients from different subgroups. IgA, IgG, and C4 levels increased significantly with patient age (P<0.01, **Table 4**). No obvious difference was noted among different TNM stages, different treatments and patients with or without metastasis.

Discussion

Our research systematically evaluated the immune function of lung cancer patients from different subgroups (age, TNM stage, and treatments) by analyzing T lymphocyte subsets and immunoglobulins. Our results indicated that

Table 4. Serum immunoglobulins and complement factors in lung cancer patients from different subgroups

Group	n	IgA	IgG	IgM	C3	C4
Age (years)						
<50	21	199.86±76.50	1096.67±214.3	139.90±69.54	112.38±20.42	24.24±9.27
>50 and <70	97	242.29±85.41	1189.93±415.80	128.24±62.10	116.32±29.14	30.71±19.39
>70	17	290.71±143.96**	1413.35±369.90**	230.88±134.22	119.35±33.40	39.59±30.27**
Treatment						
Untreated	14	248.23±87.08	1247.85±392.67	114.69±48.76	114.15±33.48	41.31±33.58
Operation	43	244.19±74.64	1206.37±304.27	126.88±55.39	109.70±33.38	31.88±23.66
Chemoradiotherapy	78	237.47±106.72	1157.12±284.22	156.62±29.77	119.37±23.73	28.55±14.29
TNM						
I	62	236.60±80.14	1204.16±473.47	125.63±57.24	112.56±33.06	32.63±24.00
II	15	232.13±79.31	1166.27±234.36	108.13±60.76	120.47±30.70	26.67±4.70
III~IV	58	249.83±113.93	1212.55±330.91	170.53±39.61	118.72±21.71	29.97±18.13
Metastasis						
No	80	237.60±86.09	1198.14±425.90	119.04±56.38	114.04±31.48	32.77±23.79
Yes	45	250.16±113.03	1214.38321.90	190.84±50.66	120.18±20.69	26.93±8.65
P		0.47	0.82	0.17	0.18	0.11

**: P<0.01 Compared with age <50.

the immune markers presented obvious alterations during aging. The frequency of $CD4^+$ T cells and the $CD4^+/CD8^+$ ratio decreased significantly as age increased, whereas IgA, IgG, and C4 levels and the frequency of $CD8^+$ T cells increased significantly as age increased.

During recent years, age was reported as the most important risk factor for tumorigenesis. The incidence and prevalence of cancer increases as age increases, which suggests a close association between aging and cancer [10]. With advanced age, numerous physiological systems change, including the immune system. Several alterations occur in both arms of the immune system with aging. The age-related alterations in the immune system entail an increased susceptibility to developing infectious diseases, cancer, Alzheimer's disease, osteoporosis and autoimmunity [11]. Our previous study demonstrated that CRP levels were significantly reduced in the young and middle age groups of lung cancer patients compared with older lung cancer patients [12]. Our results indicate that the immune function of lung cancer patients is reduced as age increases, which further verifies the correlation between age and immune function in lung cancer.

Currently, greater than 60% of newly diagnosed cancer patients and greater than 70% of cancer-related deaths occur in subjects older than 65 years [3]. The alterations occurring within the immune system during aging are known as immunosenescence. Saavedra et al reported that $CD4^+$ T cells, $CD8^+$ $CD28^-$ T cells and the CD4/CD8 ratio are useful as predictive biomarkers of CIMAvax-EGF vaccine efficacy in non-small cell lung cancer immunosenescent patients [13, 14].

We found that immune function decreased with aging, and this process may be referred to as immunosenescence in lung cancer patients. The reduced frequency of $CD4^+$ T cells indicates that the anti-tumor immunity function decreases during aging. The increased frequency of $CD8^+$ T cells indicates that inhibition of the body's immune response in anti-tumor immunity function is reduced during aging. The $CD4^+/CD8^+$ ratio also decreased, indicating that immune function decreases during aging. This phenomenon also benefits tumor proliferation. Increases in immunoglobulins are related to the cytological characteristics of cancer, including the abnormal secretion of cancer cells or other body fluids caused by cancer cells, which trigger the humoral immune response. The final effect involves increases in some immunoglobulins [2, 15, 16]. In the context of nonspecific humoral immunity of the tumor, complement acts to attack tumor cells and assists in antibody- and cell-dependent tumor cell death. The increased levels of IgA, IgG, and

C4 observed with aging indicate that the humoral immunity function decreases during aging. Importantly, in our study, we found that both cellular and humoral immunity are reduced as lung cancer patients age, indicating that immunosenescence as a result of aging may be the endogenous cause of lung cancer.

We found that both cellular and humoral immunity were significantly altered in lung cancer patients versus controls. The frequencies of $CD3^+$ T cells and $CD4^+$ T cells, the $CD4^+/CD8^+$ ratio, and IgG and C3 levels were significantly reduced in patients versus control ($P<0.05$). In contrast, the frequencies of $CD8^+$ T cells and NK cells were significantly increased in patients. Chen and colleagues suggested that immune impairment in cancer patients is associated with various factors, such as the cancer stage and the impact of treatment [17]. Our results are consistent with this view. The frequencies of $CD3^+$ T cells and $CD4^+$ T cells in the operation group were significantly increased compared with the chemoradiotherapy group. In contrast, the frequency of NK cells was significantly decreased in all other groups. Compared with the metastasis group, the number of $CD4^+$ T cells and the $CD4^+/CD8^+$ ratio were significantly reduced in the no metastasis group. This result further verified immune impairment at different stages in lung cancer patients.

In summary, we systemically evaluated the immune function of lung cancer patients from different subgroups (age, TNM stages, and treatments). Immune function exhibited an obvious trend of reduction during aging, indicating that immunosenescence during aging may serve as the endogenous cause of lung cancer.

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Disclosure of conflict of interest

None.

Address correspondence to: Qi Xia, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory of Diagnostic and Treatment of Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou 310003, China. E-mail: xiaqi@zju.edu.cn

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