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Correlation analysis of seven autoantibodies and tumor markers in lung cancer

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Abstract: **To investigate the value of seven autoantibodies (P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE) in the diagnosis of lung cancer, and the correlation between seven autoantibodies and four traditional tumor markers (CEA, NSE, CYFRA 21-1, ProGRP) in lung cancer. From January to May in 2019, the serum of 60 newly diagnosed lung cancer patients and 57 healthy people from the Affiliated Hospital of Qingdao University was collected. Seven autoantibodies were detected by enzyme-linked immunosorbent assay (ELISA), the correlation between data and tumor markers was analyzed by Spearman test. In our study, the serum concentrations of P53, SOX2, PGP9.5, GAGE7, MAGE A1, CAGE and GBU4-5 in lung cancer group were higher than those in healthy group ($P<0.05$). Furthermore, GAGE7 was positively correlated with CEA and CYFRA21-1; GBU4-5 was positively correlated with CYFRA 21-1 ($P<0.05$). The correlation between autoantibodies and tumor markers is different in various pathological types. Moreover, in the difference TNM stages of tumors, there was a statistically significant difference in the positive rates of P53 in stages Ⅰ-Ⅱ and stages Ⅲ-Ⅳ ($P<0.05$). There were no significant differences in the positive rates of P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE in lung cancer group by age, gender, smoking, and pathological types ($P \geqslant 0.05$). These results suggest that the combined detection of seven autoantibodies and four tumor markers can improve the early diagnosis of lung cancer.**

Keywords: Lung cancer; Autoantibodies; Tumor markers; Early diagnosis; Correlation analysis

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1. Introduction

Lung cancer is one of the most common malignant tumors. Surveillance, Epidemiology and End Results (SEER) of the National Cancer Institute showed that the incidence rate of lung cancer in the United States was 54.9/10 million in 2019, the incidence rate of lung cancer was 12.9% of all tumor incidence rate, mortality rate was 23.5% of all tumor mortality, and overall 5-year survival rate was 19.4%[1]. Lung cancer is invasive and heterogeneous [2, 3], intra-tumor diversity will be formed in the process of cancer development which is the main reason for the low detection sensitivity. With the early detection of the disease, the survival rates increased significantly. The 5-year survival rates of stage Ⅳ, ⅢB, ⅢA, ⅡB, ⅡA and ⅠB increased from 2% to 7%, 19%, 25%, 36% and 43%, 5-year survival increased to 50% in stage IA [4]. Therefore, early detection and immediate treatment are considered as the main measures to reduce lung cancer mortality and improve 5-year survival rates. At present, the methods of lung cancer screening mainly include imaging methods, blood molecular markers and so on. The detection of blood molecular markers includes circulating tumor cell, tumor markers, lung tumor-associated autoantibodies (TAABs), etc. With the gradual application of lung cancer autoantibody detection, the correlation between autoantibody detection and tumor markers has occurred. This work studied the difference of serum concentrations of seven autoantibodies (P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE) in lung cancer. Through the correlation between seven autoantibodies and traditional tumor markers (CEA, NSE, ProGRP, CYFRA 21-1), the diagnostic value of seven kinds of autoantibodies for lung cancer was explored.

2. Materials and methods

2.1. Research objects

From January to May in 2019, 60 lung cancer patients newly diagnosed and confirmed by pathology in Affiliated Hospital of Qingdao University were selected as lung cancer group, and 57 healthy people as healthy group. Selection criteria of lung cancer group: All patients were confirmed as lung cancer by

pathological examination[5]; All patients were given seven autoantibodies and collected four tumor markers (CEA, NSE, CYFRA 21-1, ProGRP) detection results in the same period; All patients did not receive radiotherapy, chemotherapy and surgery, and did not suffer from other diseases. Lung cancer group had 29 male patients and 31 female patients with an average age of 58.58 years old. According to the pathological types, there were 47 cases of lung adenocarcinoma (LUAD), 8 cases of lung squamous cell carcinoma (LSQCC) and 5 cases of small cell lung carcinoma (SCLC) in lung cancer group [5]. There were 29 males and 28 females in the healthy group with an average age of 51.86 years old. The healthy group did not suffer from acute or chronic diseases. This study was implemented after review and approval by the Ethics Committee of the Affiliated Hospital of Qingdao University, and all subjects had signed an informed consent.

2.2. Methods

5 mL of fasting venous blood was collected from the subjects, and the serum was separated and stored at -80℃ for centralized detection. Seven lung cancer autoantibodies (P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE) were detected by enzyme-linked immunosorbent assay (ELISA). 7-TAABs detection positive reference value: P53≥ 13.1 U/mL, SOX2≥10.3 U/mL, PGP9.5≥11.1 U/mL, GAGE7 ≥ 14.4 U/mL, MAGE A1 ≥ 11.9 U/mL, CAGEI ≥ 7.2 U/mL, GBU4.5 ≥ 7.0 U/mL is "positive", otherwise it is "negative".

2.3. Statistical analysis

All data were recorded and analyzed by SPSS Version 24.0 and indexes were tested by Shapiro-Wilk. The measurement data that do not conform to the normal distribution are represented by median and 25-75 percentile [M(P25-P75)]. The comparison between the two groups of measurement data was obtained Mann-Whitney U test, the comparison between multiple groups adopts Kruskal-Wallis H test. χ^2 test is used for comparison between count data groups. Spearman test was used for correlation analysis. $P<0.05$ indicates that the difference is statistically significant.

3. Results

3.1. Expression level of seven autoantibodies in lung cancer group and healthy group

The levels of seven autoantibodies (P53, SOX2, PGP9.5, GAGE7, MAGE A1, CAGE and GBU4-5) in lung cancer group were significantly higher than those in healthy group (Z=2.301, 2.146, 2.010, 2.721, 2.031, 2.187, 2.007; all $P<0.05$) (Table 1).

Table 1. Expression level of seven autoantibodies in lung cancer group and healthy group

project	Lung cancer group	Health Group	*Z*	*P*
P53	1.25(0.60-9.00)	1.00(0.10-3.20)	2.301	0.021
SOX2	2.10(1.13-4.93)	1.00(0.10-4.00)	2.146	0.032
PGP9.5	0.50(0.10-3.70)	0.10(0.10- 4.70)	2.010	0.044
GAGE7	3.10(1.60-5.38)	2.20(0.15-3.70)	2.721	0.007
MAGE A1	0.40(0.10-1.20)	0.10(0.10-0.25)	2.031	0.042
CAGE	0.90(0.10-2.85)	0.20(0.10-0.90)	2.187	0.029
GBU4-5	1.45(0.50-3.50)	0.10(0.10-4.20)	2.007	0.045

3.2. The relationship between the clinical and pathological characteristics of seven autoantibodies in lung cancer patients

In the TNM stages of tumors, there was a statistically significant difference in the positive rates of P53 in stage Ⅰ-Ⅱ and stage Ⅲ - Ⅳ (χ^2=6.41, $P<0.05$). There was little difference in PGP9.5, SOX2, GAGE7, GBU4-5, MAGE Al and CAGE ($P\geq0.05$), the difference was not statistically significant. There was little difference in the positive rates of seven autoantibodies (P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE) in age, gender, smoking and pathological types in lung cancer group, the difference was not statistically significant ($P\geq0.05$) (Table 2).

3.3. Correlation analysis between autoantibodies and tumor markers in lung cancer patients

In lung cancer patients, GAGE7 was positively correlated with CEA, CYFRA21-1 (r=0.243, 0.194; P=0.008, 0.036), GBU4-5 was positively correlated with CYFRA 21-1 (r=0.199, P=0.032), and other autoantibodies were not correlated with tumor markers

(P≥0.05) (Table 3).

3.4. Correlation analysis between autoantibodies and tumor markers in lung adenocarcinoma group

In the lung adenocarcinoma group, NSE was positively correlated with PGP9.5, CAGE (r=0.281, 0.289, P=0.015, 0.013), ProGRP was positively correlated with MAGE A1, CAGE (r=0.249, 0.260, P=0.033, 0.025), the remaining autoantibodies have little correlation with tumor markers (P≥0.05) (Table 4).

3.5. Correlation analysis between autoantibodies and tumor markers in lung squamous cell carcinoma group

In the lung squamous cell carcinoma group, CYFRA 21-1 was positively correlated with GAGE7 (r=0.778, P=0.023), and the remaining autoantibodies had no correlation with tumor markers (P≥0.05) (Table 5).

Table 2. the clinicopathological characteristics of seven autoantibodies in patients with lung cancer

Group	Number	P53		PGP9.5		SOX2		GAGE7		GBU4-5		MAGE A1		CAGE	
		+	-	+	-	+	-	+	-	+	-	+	-	+	-
Age															
> 60	31	5	26	3	28	5	26	4	27	5	26	2	29	5	26
≤ 60	29	4	25	1	28	2	27	3	26	3	26	1	28	5	24
χ^2		0.06		0.93		1.24		0.10		0.43		0.29		0.01	
P		0.80		0.33		0.27		0.76		0.51		0.59		0.91	
Gender															
male	29	4	25	2	27	3	26	3	26	3	26	2	27	5	24
Female	31	5	26	2	29	4	27	4	27	5	26	1	30	5	26
χ^2		0.06		0.01		0.10		0.10		0.43		0.43		0.01	
P		0.80		0.95		0.76		0.76		0.51		0.51		0.91	
Smoking															
Yes	23	3	20	2	21	1	22	3	20	4	19	2	21	2	21
No	36	6	30	2	34	6	30	4	32	4	32	1	35	8	28
χ^2		0.14		0.22		2.04		0.05		0.47		1.02		1.82	
P		0.71		0.64		0.15		0.82		0.49		0.31		0.18	
Pathological types															
Lung adenocarcinoma	47	6	41	2	45	4	43	6	41	6	41	2	45	8	39
Lung squamous cell carcinoma	8	2	6	2	6	2	6	1	7	2	6	1	7	2	6
Small cell lung cancer	5	1	4	0	5	1	4	0	5	0	5	0	5	0	5
χ^2		0.91		5.12		2.17		0.72		1.73		1.27		1.40	
P		0.64		0.08		0.34		0.70		0.42		0.53		0.50	
TNM stages															
Ⅰ - Ⅱ	54	48	6	3	51	6	48	5	49	8	46	2	52	9	45
Ⅲ-Ⅳ	6	3	3	1	5	1	5	2	4	0	6	1	5	1	5
χ^2		6.41		1.07		0.16		3.04		1.03		1.91		0.00	
P		0.01		0.30		0.69		0.08		0.31		0.17		1.00	

Table 3. Correlation analysis between autoantibodies and tumor markers in lung cancer patients

Project	CEA		NSE		CYFRA 21-1		ProGRP	
	r	P	r	P	r	P	r	P
P53	0.041	0.663	0.084	0.365	0.093	0.318	-0.02	0.834
PGP9.5	0.108	0.247	0.037	0.696	-0.052	0.578	0.099	0.290
SOX2	-0.071	0.447	-0.153	0.099	-0.110	0.236	-0.084	0.368
GAGE7	0.243	0.008	0.126	0.176	0.194	0.036	-0.082	0.377
GBU4-5	0.052	0.575	0.178	0.055	0.199	0.032	-0.001	0.998
MAGE A1	0.034	0.713	-0.022	0.816	-0.152	0.101	0.117	0.209
CAGE	-0.180	0.052	0.166	0.073	0.156	0.093	0.174	0.061

Table 4. Correlation analysis between autoantibodies and tumor markers in lung adenocarcinoma group

Project	CEA		NSE		CYFRA 21-1		ProGRP	
	r	P	r	P	r	P	r	P
P53	0.017	0.884	0.184	0.117	0.039	0.744	0.110	0.351
PGP9.5	0.017	0.888	0.281	0.015	-0.198	0.090	0.043	0.713
SOX2	-0.060	0.612	0.041	0.729	-0.015	0.902	0.108	0.360
GAGE7	0.090	0.445	0.172	0.143	0.105	0.373	-0.048	0.686
GBU4-5	-0.018	0.876	0.006	0.956	0.007	0.952	0.051	0.664
MAGE A1	0.128	0.279	0.137	0.245	0.164	0.163	0.249	0.033
CAGE	-0.114	0.335	0.289	0.013	0.144	0.220	0.260	0.025

Table 5. Correlation analysis between autoantibodies and tumor markers in lung squamous cell carcinoma group

Project	CEA		NSE		CYFRA 21-1		ProGRP	
	r	P	r	P	r	P	r	P
P53	-0.167	0.693	0.571	0.139	-0.143	0.736	0.476	0.233
PGP9.5	0.524	0.183	-0.119	0.779	-0.405	0.320	0.071	0.867
SOX2	0.595	0.120	-0.333	0.420	0.143	0.736	-0.548	0.160
GAGE7	0.012	0.978	-0.036	0.933	0.778	0.023	0.228	0.588
GBU4-5	0.491	0.217	-0.515	0.192	-0.095	0.823	-0.431	0.286
MAGE A1	0.405	0.320	-0.524	0.183	00.286	0.493	0.048	0.911
CAGE	0.643	0.086	-0.286	0.493	-0.072	0.866	-0.095	0.823

4. Discussion

Lung cancer can be divided into non-small cell lung cancer (NSCLC) and small cell lung cancer. NSCLC accounts for about 85% of all lung cancers. The most common type of NSCLC is LUAD, accounting for

about 40% of all lung cancers [6, 7]. Lung cancer patients can develop a humoral immune response to their own cancer [8]. At present, it is mainly the detection of tumor markers, which focuses on the detection of tumor-associated antigen markers. The detection for serum tumor-associated autoantibodies against overexpressed, mutated, misfolded or abnormal autologous cell antigens produced by cancer cells can identify individuals with early-stage lung cancer, and can distinguish high-risk smokers with benign nodules, tuberculosis and lung cancer [9].

The results showed that the expression levels of P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE in lung cancer group were significantly higher than those in healthy group. which make it clear that the increased expression level of 7-TAABs in lung cancer can be used to distinguish lung cancer patients from non-lung cancer patients. In 2008, Chapman[10] used lung cancer autoantibodies for the detection of lung cancer for the first time. Seven tumor autoantibodies (P53, C-MYC, HER2, NY-ESO-1, GAGE, MAGE, and GBU4-5) were detected, single antibody positive rates were 5% -36%, specificity was 96% -100%, the positive rate of combined detection is 76%, and the specificity is 92%. P53 gene is one of the earliest discovered tumor suppressor genes [11]. Mutant P53 gene can cause cell transformation and canceration, make cells proliferate indefinitely, which is related to the occurrence of various cancers (including lung cancer, colorectal cancer and breast cancer)[12]. The expression products of cancer-testis genes are tumor-testis antigens (CTA). Genes encoded by tumor-testis antigens are usually only expressed in the human reproductive system, but also in various expression in tumors, including lung cancer, melanoma, bladder cancer and liver cancer [13]. GAGE, MAGE, CAGE, and GBU4-5 all belong to the tumor-testis family. In tumors, PGP9.5 increased the deubiquitination of cyclins and may lead to uncontrolled growth of somatic cells [14]. Studies had shown that SOX2 was expressed in lung cancer, breast cancer, ovarian cancer, glioma and other malignant tumor tissues, and the ability of cancer cells to metastasize to a distant place was proportional to its overexpression[15].

In this study, the positive rates of seven autoantibodies in lung cancer group was detected. In the TNM stages of tumors, there was a statistically significant difference in the detection rates of P53 in stage Ⅰ - Ⅱ and stage Ⅲ - Ⅳ, and there was no statistically significant difference in PGP9.5, SOX2, GAGE7, GBU4-5, MAGE Al and CAGE. There was no significant difference in the positive rates of seven autoantibodies (P53, PGP9.5, SOX2, GAGE7, GBU4-5, MAGE A1 and CAGE) in age, gender, smoking and pathological types in lung cancer group. Zhang found that the positive rates of different TAABs had nothing to do with age, gender and smoking history[16].This study reveals that autoantibodies are not only increased in the advanced stages of tumors, but are also valuable for the diagnosis of early lung cancer. Compared with traditional tumor markers, which are elevated in the advanced stage of lung cancer, autoantibodies are more conducive to early screening of lung cancer and improve the diagnosis rate of early lung cancer.

In this study, to discuss the relationship between autoantibodies and tumor markers in lung cancer patients, the serum concentrations of four traditional tumor markers (CEA, NSE, ProGRP, CYFRA 21-1) were collected. In lung cancer patients, GAGE7 was positively correlated with CEA, CYFRA21-1; GBU4-5 was positively correlated with CYFRA 21-1. In the lung adenocarcinoma group, NSE was positively correlated with PGP9.5, CAGE, ProGRP was positively correlated with MAGE A1, CAGE. In the lung squamous cell carcinoma group, CYFRA 21-1 was positively correlated with GAGE7. From those we can see that there was a certain correlation between autoantibodies and tumor markers in lung cancer, and the correlation between autoantibodies and tumor markers in different pathological types were different. Detection of tumor markers (CEA, NSE, ProGRP, CYFRA 21-1) against tumor-associated antigens. Most of the tumor-associated antigens produced by these tumor cells are over-expressed, mutated, misfolded, or abnormally processed proteins. They can trigger autoreactive immune responses and can produce new epitopes or improve their compatibility with major tissues complex or T cell receptor affinity to promote immune response [17]. At present, there are few studies in this area, and further research is needed to improve the sample size. Therefore, the combined detection of seven autoantibodies and four tumor markers can improve the early diagnosis of lung cancer.

5. Conclusion

In summary, lung cancer autoantibodies were valuable for early diagnosis of lung cancer. By detecting the correlation between autoantibodies and tumor markers, it was found that autoantibodies and tumor markers should complement each other in the diagnosis of lung cancer, and combined detection can improve the diagnostic efficiency. Correlation analysis between autoantibodies and tumor markers in lung cancer also has certain value for the determination of pathological type. There are still some deficiencies in this study. The small number of samples of different pathological types in this study may have an impact on the study of the positive rate of autoantibodies in pathological types. Therefore, it is necessary to further expand the sample size to study the diagnostic value of

lung cancer autoantibodies in lung cancer.

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