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Original Article

The clinical significance of anti-mitotic spindle apparatus antibody (MSA) and anti-centromere antibody (ACA) detected in patients with small cell lung cancer (SCLC)

Liming Tan[1*], Yuhong Zhang[2], Yongqing Jiang[1], Hua Li[1], Juanjuan Chen[1], Feng Ming[1], Waimei Wang[1], Jianlin Yu[1], Tingting Zeng[1], Yongjian Tian[1], Yang Wu[1*]

[1]Department of Clinical Laboratory, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, P. R. China; [2]2010 Session, The Second Clinical Medical College of Nanchang University, Nanchang 330006, P. R. China. [*]Equal contributors.

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Abstract: Purpose: The project is aimed to detect anti-mitotic spindle apparatus antibody (MSA) and anti-centromere antibody (ACA) and explore the clinical value for the diagnosis of small cell lung cancer (SCLC), providing clinical evidence for molecular studies of SCLC. Methods: 93 SCLC patients, 208 patients with other cancers and 50 healthy controls were enrolled in this study. MSA antibodies were detected by enzyme linked immunosorbent assay (ELISA). MSA, ACA and anti nuclear antibodies (ANA) were examined by indirect immuno-fluorescence (IIF). And the results were retrospectively analyzed. Results: ① the positivity for MSA and ACA by IIF assay was respectively 36.56% and 30.11% in SCLC group, higher than in other tumor groups ($P<0.01$), ② in correlative analysis, the RR (Relative Ratio) value between MSA and SCLC was as high as 12.93, 12.74, and the RR value of ACA and ANA with SCLC was respectively 4.31 and 3.48. ③ the area under ROC (Receiver operating characteristic) curve (AUC) of MSA detection for SCLC was 0.778, with medium diagnostic value. Conclusion: MSA and ACA might serve as a new marker for SCLC because of its high detection rate. These two markers may participate in the occurrence and development of SCLC, resulting from the highly strong risk. So, the study have some application value for early detection, clinical diagnosis and potential treatments of SCLC.

Keywords: Small cell lung cancer, human mitotic spindle apparatus antibodies, anti-centromere antibodies, anti-nuclear antibodies

Introduction

Small-cell lung cancer (SCLC), a type of highly malignant tumors thought to stem from primitive neuroendocrine cells in the lung, accounting for 10-15% of lung cancer, is the leading cause of cancer death in our country, even in the world [1]. The ideal situation of cancer treatment is slow progress and good prognosis, and early diagnosis makes it possible [2, 3]. Tumour markers are usually produced from cancer cells or as response to cancer [3, 4]. It was shown that repetitive nicotine exposure induces many malignant features in SCLC cells, including increased adhesion, enhanced migration, and resistance to chemotherapy [5]. Initially, SCLC patients may respond well to chemotherapy. But when after exposure to nicotine, it is inevitable that patients become resistant to cytotoxic treatment [6]. Even if regular treatment accepted, the relative 5-year survival is just 6.4%, making SCLC as the most aggressive subtype of lung cancers [7]. Current major diagnostic methods contain pathology, iconography, with low diagnosis rate and poor specificity for SCLC [2].

SCLC has a characteristic that hematogenous metastasis happens in early stage. Some studies showed that the specificity and sensitivity of autoantibodies detection is higher than tumor antigen detection for tumors [8, 9], so detecting tumor autoantibodies has important significance for improving the diagnosis and survival of SCLC. The research showed that autoantibodies were consistently detected in the sera

Table 1. The results of autoantibodies in SCLC and other cancer groups (cases %)

Groups	N	MSA	ACA	ANA
SCLC	93	34 (36.56)	28 (30.11)	23 (24.73)
LAC	62	4 (6.45)	3 (4.84)	6 (9.68)#
LSC	52	3 (5.77)	2 (3.85)	2 (3.85)
GC	27	0 (0.00)	0 (0.00)	5 (18.52)*
HC	29	1 (3.45)	0 (0.00)	1 (3.45)
IC	22	0 (0.00)	1 (4.55)	2 (9.09)#
NC	16	1 (6.25)	1 (6.25)	3 (18.75)*
Control	50	1 (2.00)	0 (0.00)	2 (4.00)

Note: SCLC *vs.* other groups, $P<0.01$; SCLC *vs.* #, $P<0.05$; but SCLC *vs.* *, $P>0.05$.

from lung cancer [10]. As a type of lung cancer, a lot of autoantibodies, such as Anti-Hu and Anti-SOX, have been found in SCLC [11]. MSA and ACA are also autoantibodies, with rare detection in cancers and no detection in SCLC. It is interesting that in our laboratory work, we found positivity of MSA and ACA was significantly higher than that in other cancer patients in the serum of patients with SCLC.

The research have showed that autoantibodies are consistently detected in the sera from lung cancer with [9]. Moreover, antinuclear antibodies may serve as markers of lung cancer [10]. As a type of lung cancer, a lot of autoantibodies, such as Anti-Hu and Anti-SOX, have been found in SCLC [11]. However, MSA and ACA are not included in these autoantibodies. In clinical laboratory work, we found positivity of MSA and ACA in the serum of patients with SCLC was significantly higher than that in other cancer patients, with details as follows.

So in the present study, our aim was to detect MSA and ACA, and explore the clinical value for the diagnosis of small cell lung cancer (SCLC), providing a promising marker for SCLC.

Patients and methods

Patients

All tumor patients were enrolled in the study, containing outpatient and inpatient cases from the Second Affiliated Hospital of Nanchang University. Data were assembled between December 2011 and December 2014. Of 93 SCLC patients, 69 were male, aged 30-82 years (mean 62 years). Of 62 lung adenocarcinoma (LAC) patients, 48 were male, aged 42-77 years (mean 64 years). Of 52 lung squamous carcinoma (LSC), 39 were male, aged 40-81 years (mean 65 years). Moreover, gastric cancer (GC) patients were 27 cases, with 18 male, aged 45-71 years (mean 56 years). Hepatic cancer (HC) patients were 29 cases, with 20 male, aged 46-68 years (mean 56 years). In addition, there were 22 intestinal cancers (IC, 14 male, aged 44-69 years, mean 58 years) and 16 nasopharynx cancer (NC, 11 male, aged 39-71 years, mean 57 years). Sera obtained from 50 healthy blood donors (31 men) for physical check up in the same hospital were also tested as negative controls, aged 27-65 years (mean 55 years). Informed consent was obtained from each participant included in the study.

Diagnostic evidence

SCLC diagnostic criteria refer to the Small Cell Lung Cancer Clinical Practice Guidelines of NCCU, USA [12].

Bringing in standard

The patients conformed to all of the following conditions were enrolled in this study: ① informed, voluntary participation, ② clear diagnosis, intact clinical, imaging and pathologic data, ③ re-evaluating all the data of patients participated, without other autoimmune diseases or other cancers, ④ in healthy volunteers control group the results of accessory examination were normal, containing complete blood count, blood pressure, blood glucose, sternum, electrocardiograph and hepatobiliary and pancreatic examination.

Ruling out standard

The patients having any of the following conditions were ruled out: ① tumor type is not explicitly or primary tumors, ② with severe heart, lung, liver, kidney and other systemic disease, Thyroid disease, diabetes, ③ women in pregnancy or lactation, ④ no consistence with the rules in bringing in standard.

Reagents and methods

Blood samples

3 ml fasting blood samples from the vein were collected in a tube without any anticoagulant. After centrifugation at 1026 g for 15 minutes, sera were separated, divided into aliquots and frozen at -20°C.

Table 2. MSA detection in patients with SCLC and other types of cancer by two methods (cases %)

Group	N	ELISA	IIF		
		MSA	MSA	ACA	ANA
SCLC	93	39 (41.94)	34 (36.56)	28 (30.11)	23 (24.73)
No-SCLC	208	11 (5.29)	9 (4.33)	7 (3.37)	19 (9.13)
RR		39 (41.94)	12.74	4.31	3.48
χ^2		11 (5.29)	54.53	11.38	14.58
p		12.93	<0.01	<0.01	<0.01

Note: RR value: highly strongly relative (10.0), strongly relative (3.0-9.0), intermediately relative (1.5-2.9).

Table 3. The clinical evaluation results in SCLC

Group	Sensibility %	Specificity %	Likelihood ratio (+)	Likelihood ratio (-)	Availability %	Youden index
MSA	36.56	95.19	7.60	0.67	77.08	0.32
ACA	30.11	96.63	8.93	0.72	76.08	0.27
ANA	24.73	89.90	2.45	0.84	69.77	0.15

Table 4. Advantage analysis (P) and consistency analysis [Kappa(κ)] between antibodies in SCLC group

Ground	MSA & ACA	MSA & ANA	ACA & ANA
P	0.327	0.052	0.383
$K_{(P<0.01)}$	0.374	0.328	0.435

Note: Kappa(κ): 0.4-0.6 as moderate consistency, 0.6-0.8, as high consistency, >0.8, as great consistency.

Operating instructions

Immunofluorescence: MSA, ACA and ANA antibodies were detected by indirect immunofluorescence with commercial kits from Euroimmun Company (Lübeck, Schleswig-Holstein, Germany). MSA were tested on snap-frozen sections of HEp-20-10 cell lines. Detection of ACA and ANA was performed on Mosaic (Hep-2 cells). 25 μl diluted samples were added on the slides, covered with sections. After incubation with 30 min at room temperature, the slides were washed. Then FITC-anti-human IgG antibodies were added with 30 min incubation. Finally, the slides were covered with glycerin, then observed by fluorescence microscope.

ELISA: MSA antibodies were detected by enzyme linked immunosorbent assay (ELISA) kits from R&D Company (Lorton, Virginia, USA). Diluted sera and controls were added into the corresponding wells, incubated for 30 min at 37°C. After washing with buffer for five times, 50 μl HRP-conjugated mitotic spindle apparatus antigens were applied for 30 minutes at 37°C. After washing as described previously, the substrate buffer was added for 10 min incubation at 37°C. Then the optical density (OD) was read at 450 nm and results were analyzed following stop buffer added.

Statistical analysis

Statistical analysis was performed using SPSS 16.0 software. Enumeration data were described in percentage. ① one-factor analysis of variance between groups was completed using χ^2 test, ② the relevance between MSA and SCLC was confirmed by relative risk (RR) and its significance, with RR>1 as positive relative risk and RR<1 as protective factor, ③ advantage analysis between antibodies was performed using paired χ^2 test. Consistency analysis between antibodies was completed by calculating kappa value (P<0.01), ④ quantitative data were analyzed using receiver operating characteristic (ROC) by calculating the area under the curve (AUC).

Evaluation indicators

According to the antibody results from the patients diagnosed by Small Cell Lung Cancer Clinical Practice Guidelines of NCCU, USA, the clinical evaluation indicators of autoantibodies were calculated, containing sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, accuracy and Youden's index.

Results

Results of autoantibodies in patients with SCLC or other cancer groups (IIF)

The positivity of MSA, ACA and ANA in SCLC group was respectively 36.56%, 30.11% and 24.73%, and for MSA and ACA, there were significant differences against other groups by χ^2 test (P<0.01), as shown in **Table 1**.

The correlation analysis between SCLC and the autoantibodies containing MSA, ACA, ANA

The positivity of MSA and ACA in SCLC group was significantly different (P<0.01) against

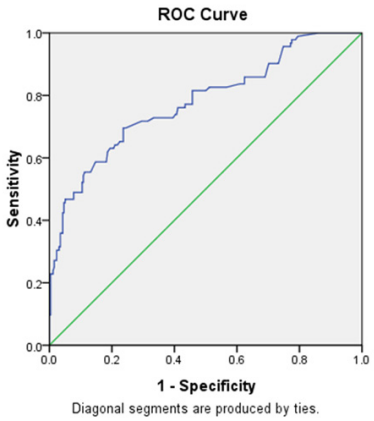

Figure 1. ROC curve of MSA for SCLC diagnosis. Note: AUC 0.5-0.7, poor diagnostic value, 0.7-0.9 moderate diagnostic value, >0.9 high diagnostic value.

Test Result Variable(s):MSA

Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.778	.030	.000	.719	.836

other cancer groups by χ^2 test. The correlation analysis showed that MSA was a highly strongly positive relative risk. Moreover, ACA and ANA was strongly positive relative risk, as detailed in **Table 2**.

The clinical evaluation indicators about MSA, ACA and ANA from SCLC patients (IIF)

In SCLC group, the specificity of MSA and ACA was respectively 95.19% and 96.63%, as listed in **Table 3**.

The advantage analysis (P) and consistency analysis [Kappa (κ)] between MSA, ACA and ANA in SCLC group

The consistency analysis showed that in SCLC group the consistency was very poor between these three antibodies, and the advantage analysis showed that there was no difference between them for SCLC diagnosis as displayed in **Table 4**.

ROC curve evaluation of MSA for SCLC diagnosis

The area under the curve (AUC) of MSA was 0.778, with moderate diagnostic value for SCLC (P<0.01) as revealed in **Figure 1**.

Discussion

SCLC comes from pulmonary neuroendocrine cells, as the most malignant lung cancer. Till now, SCLC has no efficient early diagnostic method and treatment, with high recurrence rate and 6.4% five year survival rate [13]. Studies have showed that there was close relationship between SCLC and some rare autoimmune neurologic paraneoplastic syndrome [14, 15]. Antibodies associated with neurologic syndromes, which are related to result from an autoimmune attack on neuronal tissue, stimulated by similar neuronal antigens ectopically expressed in cancer cells [16, 17]. Many autoantibodies, such as SOX-1, Hu-ab, ZIC-4, have been detected in the early SCLC patients without autoimmune diseases or patients developing into SCLC in future [18]. Although the antibody titers were low, they provided evidences that autoimmune response could impact on the development of SCLC. These immune reaction may be a window phase reaction of SCLC. That is to say the immune system has changed before appearance of SCLC, which implies that it is accessible for the early diagnosis and forecast of SCLC by detecting autoimmune markers. Proper intervention before the occurrence of SCLC and correct treatment for SCLC in early phase can improve the rate of diagnosis and survival of SCLC, and provide promising directions for clinical treatment of SCLC.

The major risk factors of lung cancer are smoking, working surroundings and heredity. Researchers have reported that contact of hazardous materials induced injury of DNA, damage of spindle and cell division arrest in the metaphase in lung [19]. When in danger, more than two daughter-cell produced or cell fusion happened, resulting in aneuploid cells, which might be related with abnormal numbers of centrosome [20]. Centrosome is an organelle that serves as the main microtubule organizing center. During the mitosis, the centrosome migrates to opposite poles of the cell. The number of centrosome is regulated by the function of mitotic spindle. When in metaphase of cell division, all chromosomes line up at the equatorial plane, and the division of chromosomes depend on the microtubules [21].

Studies have showed that one of abnormal functions related genes of mitotic spindle is the overexpression of mitotic spindle related proteins in tumor cells. The overexpressed proteins accumulate in cells. When there is dysfunction of immune tolerance, the autoimmune reaction appears, with production of autoantibodies [22]. It seems that the finding has some relationship to our job. In this study, the positivity of MSA and ACA in SCLC group was respectively 36.56% and 30.11%, higher than other tumor groups, with significant differences .We made a correlation analysis of our results. The RR value of MSA for SCLC was respectively as high as 12.93 and 12.74 using two assays. In addition, the RR value of ACA and ANA for SCLC was respectively 4.31 and 3.48. So MSA is a highly positive correlation risk factor for SCLC, and ACA and ANA are strongly positive correlation risk factors. It indicates that the immune reaction against mitotic spindle and centromere by immune system may have some impact on the occurrence and development of SCLC. And these antibodies have great clinical application value for the early diagnosis of SCLC.

Recently, there have been a few researches about the autoimmune reactions in tumor. Many autoantibodies could be detected in patients of SCLC, but no detection of anti-mitotic spindle. In this project, we selected Hep-20-10 cellular matrix to detect MSA antibodies by IIF assay. Hep-20-10 cell line has more than ten times mitotic cells than HEp-2 cell line, which is easy to observe the special structures related with division phase, such as centromere, mitotic spindle and centrosome. The specificity of MSA for SCLC was 95.19% and positivity was 36.56%, higher than other tumor groups, with great significance by χ^2 test ($P<0.01$). Using ELISA quantitative assay, MSA was tested. ROC curve of MSA detection for SCLC was made, and AUC was 0.778, with moderate diagnostic value. The division of chromosome depends on the drag of mitotic spindle against centromere. The study found that the specificity of ACA for SCLC was as high as 96.63%, and the positivity was 30.11%, with significant difference by χ^2 test ($P<0.01$) compared with other cancer groups. It indicates that the dysfunction of related mitotic spindle and centromere in cell division may be one of pathologic mechanisms of SCLC. But the proteins involved in the reaction need to be studied. Timely diagnosis can improve treatment rate and survival rate by intervening the pathways in early phase of SCLC.

The positivity of MSA and ACA in SCLC group was higher than that in other tumor groups. But the reason remains unknown. In cell division, spindle fibers of mitotic spindle link to the centromere and drag the centromere to separate the sister chromosome. Consistency analysis showed that there was poor consistency between MSA and ACA in SCLC group. It indicates that in SCLC, MSA and ACA are not the simple autoantibodies only against the mitotic spindle and centromere. The autoimmune responses appear because that the immune system fails to distinguish self antigens. Inner changes in tumor cells can lead to some new antigens, but many antigens are not unique to tumor cells. Another type of antigens are produced following disorders of some related proteins during the occurrence and development of tumors. The complicated mechanism of regulating cell division by the mitotic spindle and centromere results in abnormal function of antigenic proteins regulating division. The type and mechanism of those proteins may be diverse, so how MSA and ACA autoantibodies are produced needs us to explore. And to clarify this problem is also valuable for treatment of SCLC.

The target antigens of ANA exist in a whole cell, containing the nucleus and cytoplasm. ANA are positive in many diseases, with poor positivity. In this study, we found that in SCLC group the positivity of ANA was 24.73%, with significant differences by χ^2 test ($P<0.05$) compared with other types of cancer. Although ANA has no advantage in differential diagnosis of cancers, it is valuable for differential diagnosis between several types of lung cancers.

In conclusion, MSA and ACA are highly detected in SCLC, making it possible that they might be used as potential markers. They are positively risk factors of SCLC, and may participate in the occurrence and development of SCLC. So, the combination of MSA and ACA has some application value for early diagnosis, clinical diagnosis and promising treatments of SCLC.

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Disclosure of conflict of interest

None.

Address correspondence to: Liming Tan, Department of Clinical Laboratory, The Second Affiliated Hospital of Nanchang University, Nanchang 330006, P. R. China. Tel: +86-791-86300410; E-mail: yuuje@126.com

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