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Original Article

Application of serum NY-ESO-1 antibody assay for early SCLC diagnosis

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Abstract: Background: NY-ESO-1 antibody is one of the cancer-related antibodies. The purpose of this study was to investigate the diagnostic role of the NY-ESO-1 humoral immune response in small cell lung cancer (SCLC). Methods: We recombined the recombinant protein of NY-ESO-1 antibody and NSE, analyzed them by Enzyme-linked immunosorbent assay, and then established the Receiver Operating Characteristic (ROC) curve to estimate the diagnostic value of NY-ESO-1 antibody, NSE and their combinations. Results: According to detection, the positive rate of NY-ESO-1 humoral immune response (26.3%), NSE (43.8%) and their combinations (10.5%) were all lower than the negative rate which indicated that the NY-ESO-1 antibody might be down-regulated in SCLC. And the positive rate wasn't related to clinicopathologic characteristics. The ROC curve demonstrated that with a 37.17% sensitivity and a 91.7% specificity along with a AUC of 0.619 for NY-ESO-1ab as well as with a 48.3% sensitivity and a 90.87% specificity along with a AUC value of 0.773 for NSE, their diagnostic value were both high. Besides, the diagnostic value of their combinations was also good for a AUC of 0.83 and a 69.12% sensitivity and a 91.8% specificity. There were significant difference of diagnostic value among three types above (NY-ESO-1 vs. NSE, $P < 0.01$; The Combinations vs. NY-ESO-1, $P < 0.0001$; and the Combinations vs. NSE, $P<0.04$). Conclusion: In conclusion, NY-ESO-1ab, NSE and their combinations all were important diagnostic markers for SCLC. Moreover, the diagnostic value of their combinations was higher than any single of them. And NY-ESO-1 humoral immune to NSE might be a potential diagnostic indicator in SCLC.

Keywords: NY-ESO-1, SCLC, diagnosis

Introduction

Small cell lung cancer (SCLC), a type of highly malignant cancer accounts for about 20% of lung cancers [1-3]. Even the positive response to a certain extent found in patients, the current chemotherapy and radiotherapy failed to achieve dramatically improvement of overall survival (OS). Due to the fact that SCLC has a tendency to be easily and widely disseminated when it occurs, the 5-year survival rate is low [4, 5]. Therefore, the early diagnosis would be crucial for improvement of the long term survival of SCLC patients.

The detection of autoantibodies to tumor-associated antigens could be made prior to the presence of symptomatic disease in many tumors [6-10], would strikingly facilitate the early diagnosis of cancers. Caroline et al screened relevant autoantibodies in patients with SCLC and found the presence of an autoantibody to one or more cancer-associated antigens might provide an important additional help to the early diagnosis of SCLC [11]. NY-ESO-1 antigen is one of cancer/testis (CT) antigen. It was originally identified in esophageal cancer and has been shown to be strong immunogenic [12]. Spontaneous NY-ESO-1 antibodies are often observed in 52% of prostate cancer patients, 7.7-26.5% of breast cancer patients, 4.2-20.0% of lung cancer patients, and 9.4% of melanoma patients [13-17]. However, they were almost not detected in noncancerous donors [18]. Therefore, the NY-ESO-1 humoral immune response is considered to be a serological marker on detecting those cancers mentioned and facilitating the clinical

management [19]. Although the occurrence of NY-ESO-1 had been studied in ling cancer in previous studies [17], the diagnostic value of it was rarely reported.

In our study, we aimed to investigate the feasibility of an assay combining the NY-ESO-1 antibody with NSE in early diagnosis of SCLC.

Materials and methods

Blood samples and patient details

This study was conducted at Chinese PLA General Hospital and permitted by the Ethic Committee of the hospital. 57 serum samples were collected from patients either with biopsy-proven SCLC or with a characteristic Paraneoplastic Neurological Disorders (PND) if further follow-up investigations revealed SCLC. Meanwhile, 47 serum samples were obtained from patients without SCLC and other lung cancer (including 24 patients with benign pulmonary diseases (BPD) and 23 patients with no specific pulmonary diseases (NPD)) matching with age and gender as control. All patients had signed written informed consent in advance. All serum samples were put into blood collection tube of EDTA after extracting and stored at -70°C for using.

Enzyme-linked immunosorbent assay for NY-ESO-1 antibody and NSE

After recombining the NY-ESO-1 protein as described in previous study [20], the Enzyme-linked immunosorbent assay was conducted. Firstly, 100 μl of 1 μg/ml recombinant protein in coating buffer was loaded to each well of a 96-well PolySorp immunoplate (Nunc, Denmark), and incubated overnight at 4°C. The plates were then washed with PBS and blocked with 100 μl per well of 5% BSA/PBS for 2 h at room temperature. After washing with PBS again, 100 μl/well of serially diluted serum in PBS/2% BSA were added into each well and incubated for 2 h at room temperature. Then, after wash extensively with PBS, goat anti-human IgG (A8792-2ML, Sigma Chemical Co, China) as a secondary antibody was added to the wells and incubated for 1 h at room temperature. Washing the plates, 50 μl per well of HRP chromogenic substrate (SureBlue TMB, Xin Xingtang, Biotechnology Co, China) was put into the plates and developed signals for 30 min at room temperature. The absorbance was read at 620 nm if blue was showed before adding the stop solution, or at 450 nm if yellow was showed after adding the stop solution using an enzyme-linked immunosorbent assay (ELISA) reader (Benchmark Microplate Reader; Bio-Rad, Hercules, CA, USA). The levels of NY-ESO-1 humoral response were assessed with optical density (OD) values, and the levels of NSE were measured with Access NSE test kits (Roche, Inc, Switzerland) of E601 Immunoassay System.

Statistical analysis

All statistical analysis were performed with IBM SPSS Advanced Statistics 19.0 and MedCalc version 9 (MedCalcSoftware Broekstraat 52, B-9030 Mariakerke, Belgium). Parson × 2, continuity correction × 2 and Fisher's exact test were used to assess the associations between NY-ESO-1 antibody expression and pathological parameters. ROC curve was generated to evaluate the diagnostic values of NY-ESO-1, NSE and their combinations. Logistic regression analysis, with the presence of lung cancer as the binary dependent variable and the levels of the markers as the predictor variables, were performed to calculate predicted probability values from the combinations of markers. Correlations among the levels of those markers were evaluated with Spearman rank tests. For all statistical analysis, *P* value less than 0.05 was regarded as significant.

Results

Detection of NY-ESO-1 humoral immune response positivity

After examining the reactivity of serum samples from 47 controls to NY-ESO-1 recombinant protein, their OD value were ranged from 0.007 to 0.137 and the cut off value was 0.083. Therefore, the NY-ESO-1 humoral immune response was defined as positivity, when the OD value was higher than 0.083.

Relationship between NY-ESO-1 humoral immune responses and clinicopathologic characteristics of SCLC patients

Serum samples were obtained from 57 SCLC patients, including 18 LD, 39 ED patients at admission (**Table 1**). The NY-ESO-1 antibody was detected in 38.9% (7 of 18) of LD, 20.5% (8 of 39) of ED SCLC patients, and the overall detection rate was 26.3% (15 of 57). The sub-

Table 1. Relationship between NY-ESO-1 antibody positive rate and demographic features in SCLC

Variables	NY-ESO-1Ab		*P*-value
	Positive (n)	Negative (n)	
Gender			0.371
Male	14	33	
Female	1	9	
Age (Years)			1.00
> 65	3	7	
≤ 65	12	35	
Smoking history daily quantity			0.142
> 20	7	27	
≤ 20	8	15	
Time (year)			1.00
≥ 20	11	30	
< 20	4	2	
Stage			0.38
LD-SCLC	7	10	
ED-SCLC	8	32	
Stage (TNM)			0.138
I	5	6	
II	8	24	
III	1	8	
IV	1	4	
Distant metastasis			0.986
Negative	10	30	
Positive	5	12	

Table 2. Frequency of NY-ESO-1 positive and NSE positive in SCLC patients

Markers positive	SCLC patients (n = 57)		Controls (n = 47)
	LD-SCLC (n)	ED-SCLC (n)	BPD and NPD (n)
NY-ESO-1ab only	4	5	1
NSE only	5	14	3
NY-ESO-1ab and NSE	3	3	0
None	6	17	43
Total	18	39	47

group analysis of the SCLC patients based on patient's characteristics showed that NY-ESO-1 antibody positive rate was not significantly associated with gender, age, smoking history, Stage, distant metastasis and tumor progression (**Table 1**).

Frequencies of NY-ESO-1 humoral immune responses and conventional tumor markers in all groups

The number of SCLC patients positive for one marker or two markers was showed in **Table 2**. Among the 57 patients with SCLC, 25 (43.8%) patients were NSE positive while 15 (26.3%) patients were NY-ESO-1 positive. Among them, the number of patients who were both positive to NSE and NY-ESO-1ab was 6 (10.5%) including 3 LD-SCLC and 3-ED-SALC, the number of patients who were only positive to NSE was 19 (33.3%) including 5 LD-SCLC and 14 ED-SCLC and who were only positive to NY-ESO-1ab was 9 (13.2%) including 4 LD-SLSC and 5 ED-SCLC. Meanwhile, 23 of the 57 (40.35%) patients including 6 LD-SCLC and 17 ED-SCLC were negative for both NSE and NY-ESO-1ab.

Diagnostic values of NY-ESO-1 humoral immune responses and NSE in SCLC patients

The diagnostic values of NY-ESO-1 humoral immune responses and NSE as well the combination of them were analyzed via ROC curve. For NSE positive, the AUC was 0.773 combining a sensitivity of 37.17% and specificity of 91.7%. For NY-ESO-1 positive, the AUC was 0.619, and the sensitivity and specificity were 48.3% and 90.87%, respectively. Combination of two markers gave even higher AUC value as 0.83, corresponding with a sensitivity of 69.12% and specificity of 91.8%. The cut-off value of three types of ROC curves were 26.66 ng/mL for NSE, 23.22 ng/mL for NY-ESO-1 and 12.5 ng/ml for the combination. The ROC curve was displayed in **Figure 1**.

Correlations between the concentrations of NY-ESO-1 and NSE

Levels of NY-ESO-1 and NSE were not well correlated. The Spearman's coefficient of rank correlation rho was -0.102 (95% CI = -0.441 to 0.261, **Figure 2**). The associated *P* value is 0.584 and therefore suggests no significant relationship between the two variables.

Discussion

The potential value of using a panel of autoantibodies for the early detection and monitoring of cancer has been shown in the previous publications [12-17, 19]. Prior to the onset of symp-

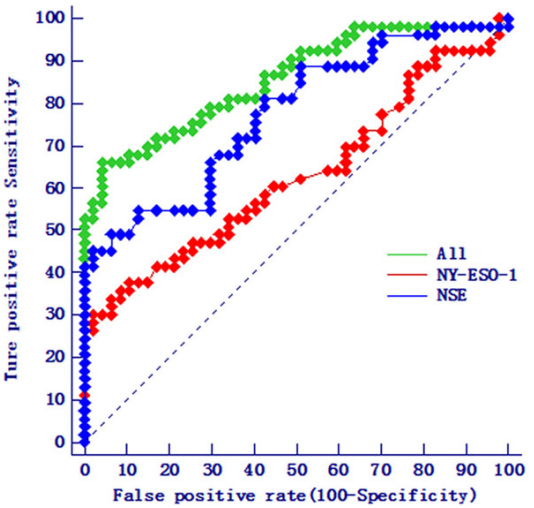

Figure 1. The ROC curve of NY-ESO-1, NSE and their combinations diagnostic value in SCLC. The AUC value of them were 0.619, 0.773, and 0.83, corresponding with a sensitivity and specificity of 37.17%, 91.70%, 48.30%, 90.87%, 69.12%, and 91.8% Respectively.

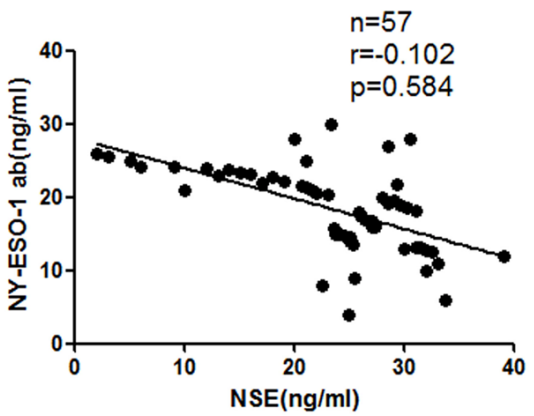

Figure 2. The correlation between NY-ESO-1 and NSE. Spearman's coefficient of rank correlation r was -0.102, and the *P* value was 0.584.

toms of cancer [13, 18], some autoantibodies to relevant tumor associated antigens have already been detected. Autoantibodies have also been observed up to 5 years before lung cancer confirmed by CT screening [21-25], indicating tumor associated antibodies detected before clinical presentation would be critical to the early diagnosis of lung cancer.

NY-ESO-1 antibodies are a kind of cancer related antibody and has been reported to be expressed in various malignancies. The NY-ESO-1 and the combination of it with other genes were considered to be important marker to increase the rate of early detection in tumor. In our study, the NY-ESO-1 humoral immune responses were analyzed which manifested that the positive rate of patients with SCLC to NY-ESO-1 was 26.30% and to NSE was 43.8% while those of the healthy controls were 1.75% and 5.26%. Meanwhile, the positive rate of the combinations of NY-ESO-1 and NSE (10.5%) was also higher in the patients with SCLC than in the healthy controls (0%). These might indicated that both NY-ESO-1ab and NSE were decreased in SCLC and they might be useful for the early detection of SCLC. Besides, previous studies reported the positive rates of NSE were 62%-88% in ED SCLC but only 42%-67% in LD. In our study, the positive rate of NSE in LD-SCLC and ED-SCLC were 44.44% and 43.59%. The positive rate of NY-ESO-1ab was 38.9% in LD SCLC patients, and 56.41% in ED-SCLC. The combinatorial use of the NY-ESO-1 antibody and NSE showed a positive rate of 16.67 % and 7.69% in patients with LD-SCLC and ED-SCLC, severally.

Next, we estimated the relationship of NY-ESO-1ab and clinicopathologic characteristics, the outcome told that there was no association between them. However, whether NY-ESO-1ab participated in the development of SCLC is still uncertain and need further to explore. Then we measured the effects of NY-ESO-1ab, NSE and their combinations on the diagnosis of SCLC. The result showed that as stand-alone marker for screening lung cancer, the sensitivity and specificity for NY-ESO-1 (Sensitivity: 37.17%, Specificity: 91.70%) and NSE (Sensitivity: 48.30%, Specificity: 90.87) were both very low while their AUC were 0.619 and 0.773, respectively. In addition, the diagnostic value of their combinations also exhibited very high with a AUC of 0.83 and the sensitivity and specificity were 69.12% and 91.8% respectively. This method of combining markers could be a plausible approach to the low-positivity issue of conventional markers in screening for SCLC. The AUC values of the 2 markers and Their Combinations were significantly different from each other. As the levels of NY-ESO-1ab were not well correlated with NSE, thus NY-ESO-1 could be an independent marker for predicting SCLC, and this supports the benefit of the strategy of combining markers.

This is the first study that compared the diagnostic performance of NY-ESO-1 with those of other markers using quantitative determination

of marker concentrations and ROC analysis. Although the positive rate of NY-ESO-1ab was lower than NSE, the combination of two makers could significantly enhance the chance of identifying SCLC. The outcome suggested SCLC could be detected by using a combined assay of markers that include an NY-ESO-1ab with a high sensitivity and specificity. Since the number of patients in the study was limited, the further large-scale studies with a prospective should be designed, an automated platform to measure NY-ESO-1ab levels would facilitate the application of the assay of NY-ESO-1ab combined with conventional markers in early diagnosis of SCLC.

In conclusion, we found that NY-ESO-1 antibody reduced in SCLC. It may be an independent indicator in the diagnosis of SCLC.

Disclosure of conflict of interest

None.

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