Serum human epididymis protein 4 (HE4) may be a better tumor marker in early lung cancer

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Abstract

Background: Human epididymis protein 4 (HE4) had been shown to be an ideal biomarker in ovarian cancer. However, there were fewer reports on its application in lung cancer. We explored the diagnostic value of serum HE4 as a biomarker in early lung cancer by comparing it with other biomarkers.

Methods: 162 individuals including 112 cases of lung cancer at early stage and 50 healthy people as controls were enrolled. The serum concentrations of biomarkers were determined with the Roche Elecsys assays.

Results: In comparison to the other biomarkers such as carcinoembryonic antigen (CEA), neuron-specific-enolase (NSE), serum cytokeratin 19 fragment (CYFRA 21-1) and progastrin-releasing peptide (proGRP), serum HE4 was one of the biomarkers with the highest sensitivity (43.8%) and specificity (95.0%) for early lung cancer diagnosis. In terms of histological results, serum HE4 was the best biomarker both in adenocarcinoma (AC) and squamous carcinoma (SC), and serum proGRP was the best in small cell lung cancer (SCLC). The combination of proGRP, NSE and HE4 could determine the histological type of lung cancer with a very high accuracy of 93.8%.

Conclusions: These findings suggested that serum HE4 was a better biomarker in early lung cancer than other frequently-used biomarkers.

Keywords: lung cancer; HE4; tumor marker; early diagnose

1 Introduction

Lung cancer is the leading cause of cancer death and shows high incidences in both men and women worldwide. It is estimated that > 20% of Chinese cancer patients died of pulmonary cancer [1-3]. As one of the most aggressive diseases most of lung cancer were within advanced stages and lost the opportunity to undergo surgery by the time of diagnosis [4]. Both The National Academy of Clinical Biochemistry (NACB) and European Group on Tumor Markers (EGTM) recommended carcinoembryonic antigen (CEA), neuron-specific-enolase (NSE), serum cytokeratin 19 fragment (CYFRA 21-1) and progastrinreleasing peptide (proGRP) as biomarkers for differential diagnosis, prognosis, monitoring therapy, detection of recurrent disease in their practice guidelines [5]. However, all of these biomarkers were not recommended for diagnosis of lung cancer at early stage. Therefore, it is urgent to search sensitive and specific biomarkers to screening lung cancer, especially in the early stage of the disease[6].

Human epididymis protein 4 (HE4) is a product of the whey-acidic-protein 4-disulphide core domain 2 (WFDC2) gene which expresses in a variety of normal tissues such as respiratory tract and genital tract especially in ovary [7,8]. However, HE4 is mostly demonstrated as a biomarker for ovarian cancer, and the combination of HE4 and CA125 provided a powerful tool for diagnosis, monitoring and prognosis in ovarian carcinoma [9-11]. Compared to CA125, HE4 elevated significantly in cases at early stage. Recently, scholars found that HE4 could also positively express in lung cancer cells, and high concentration HE4 could be detected in serum and tissue of patients with lung cancer [12-14].

2 Materials and methods

2.1 Patients

The study approval was obtained from the ethical committee of Shandong Cancer Hospital & Institute. All the patients were in hospital from May 2014 to July 2015. In this study, we enrolled 112 patients with pulmonary carcinoma at early stage, and the women of them had no history of gynecological diseases. Their blood samples were collected the day before operations. Fifty healthy donors whose composition of gender and age were matched to the patient group were selected as healthy control (Table 1). The serum creatinine concentrations of both patients and controls were examined to disqualify those with abnormal tumor marker concentrations due to renal failure.

Based on the post-operation pathologic or EBUS-TBNA reports, they included 46 cases of adenocarcinoma (AC), 35 cases of lung squamous carcinoma (SC) and 31 cases of small cell lung cancer (SCLC). NSCLC and SCLC were staged according to the Tumor Node Metastasis (TNM) classification system developed by the American Joint Committee on Cancer (AJCC) and a modified version of the Veterans Administration Lung Cancer Study Group (VALSG) staging system, respectively [15,16]. Those with NSCLC, and 62 cases were at stage I and 19 at stage II. The patients with SCLC were at local limited disease stage.

2.2 Specimen collection and measurements

About 3 ml of peripheral blood was collected from each case in coagulated tube. The serum was separated by centrifuging at 1600×g for 10 min, and then transferred to a new

Eppendorf tube, and stored at -70°C until further analysis. The concentrations of CEA, NSE, Cyfra21-1, proGRP and HE4 were measured with electrochemiluminescence assays in a Roche E601 Immunoassay Analyzer according to the manufacturer's instructions.

2.3 Statistical analysis

All statistical analyses were performed by using SPSS 17.0 software. The concentrations of serum HE4 and other tumor biomarkers in the different groups were compared using the Kruskal-Wallis test, and the data were reported as median (range) because of their abnormal distribution. Significant differences were accepted at a $p < 0.05$. Receiver operating characteristic (ROC) curve was drawn according to concentrations of biomarkers between cancer groups and control groups. Binary logistic regression model was used to establish the diagnostic model and evaluate the value of biomarkers in histology differential diagnosis.

3 Results

3.1 The serum concentration of HE4 and other biomarkers in lung cancer patients and healthy controls

The serum concentration of CEA, NSE, Cyfra21-1, proGRP and HE4 in lung cancer patients and healthy controls were presented in Table 2. In order to illustrate vividly, Fig. 1 was their scatter diagram (the ordinate was logarithmic to reduce data fluctuation). Higher

concentrations of serum HE4, NSE and Cyfra21-1 were found in lung cancer patients than in healthy controls ($p<0.0001$, $p=0.001$ and $p<0.0001$, respectively). In addition, it was observed that, HE4 and Cyfra21-1 had the best results to distinguish between the patients with lung cancer and the healthy controls. The comparison between any two subgroups of NSCLC, SCLC and healthy were also performed. HE4, proGRP and Cyfra21-1 showed statistical difference between any two subgroups of NSCLC, SCLC and healthy. However, serum CEA was found different only between NSCLC and healthy controls and NSE only between SCLC and healthy controls, as shown in Fig. 1.

3.2 The diagnostic value of serum tumor biomarkers for early lung cancer

In order to evaluate the diagnostic value of HE4 and the other tumor markers in early lung cancer, ROC analysis was performed. The area under ROC (AUC-ROC) of HE4, CEA, NSE, Cyfra21-1 and proGRP for discriminating lung cancer from healthy controls was 0.821 (95% CI, 0.754–0.887), 0.598 (95% CI, 0.507-0.689), 0.660 (95% CI, 0.570-0.750), 0.699 (95% CI, 0.613-0.785) and 0.504 (95% CI, 0.416-0.592), respectively. The cut-off value with a specificity of 95% were 66.8 pmol/l for HE4, 5.89 μ g/l for CEA, 16.5 μ g /l for NSE, 3.79 μ g/l for Cyfra21-1 and 73.5ng/l for proGRP, respectively. The particular ROC curves of serum HE4 and other biomarkers were depicted in Fig. 2. Furthermore, we concluded that HE4 had the largest AUC and it was the best one to diagnose early lung cancer.

For the reason that most of biomarkers in lung cancer were histological type related, we analyzed them in SC, AC and SCLC respectively. The results demonstrated that serum HE4

was the best diagnostic marker for AC (ROC-AUC: 0.868; 43.50% sensitivity and 95.00% specificity) and SC (ROC-AUC: 0.863; 57.10% sensitivity and 95.00% specificity), as shown in Table 3(B) and (C). However, serum proGRP was the best biomarker for SCLC (ROC-AUC: 0.848, 74.20% sensitivity and 95.00% specificity). (Table 3D)

3.3 The combination of biomarkers in diagnosis of early lung cancer

In order to increase the sensitivity of biomarkers in diagnosis of early lung cancers, the biomarkers were combined. Binary logistic regression analyses were applied to screen the most valuable tumor markers and establish the diagnostic model. For lung cancer patients, the diagnostic model based on regression equation owned a sensitivity of 51.8% and a specificity of 95%, which was slightly better than that of the combination of HE4 and proGRP (2 best markers). The equation was $P = 0.213 \times \text{NSE} + 0.570 \times \text{Cyfra21-1} + 0.099 \times \text{HE4} + 0.011 \times \text{proGRP} - 9.343$ (Fig. 2A). The combination of 2 best markers could improve the Youden's index in AC, SC and SCLC, respectively, and is shown in Table 3.

3.4. Application of biomarkers in differential diagnosis of histology

The histological type is important for the doctors to deal with lung cancer properly. Since above serum biomarkers were elevated in a histology-related fashion, we explored the possibility of identifying histological type depended on them. As shown in Table 4, proGRP

alone could distinguish NSCLC from SCLC with an accuracy of 90.2%, and the combination of proGRP, NSE and HE4 could improve the accuracy rate to 93.8%. It implied that proGRP, NSE and HE4 could be used to predict the histological type in those hard to get tissue for biopsying.

4. Discussion

As early as 2006, Bingle et al. had demonstrated that the expression of HE4 protein could be analyzed in lung cancer [17]. Subsequently, scholars found that HE4 were positively expressed in some lung cancer cells, and high concentration HE4 could be detected in serum and tissue of some patients with lung cancer. But up to now, there were fewer reports comprehensively introducing its application in diagnosis of early lung cancer. In present study, we measured the serum concentration of HE4 in 112 early lung cancer patients and found that HE4 increased in 43.8% patients with early lung cancer. It is about as high as 2-fold of any other frequently-used biomarker. It indicated that HE4 could be a better biomarker for diagnosis of lung cancer at early stage.

The diagnostic ability represented by the ROC-AUC in this study is not exact match with other's reports. Our results are similar to Yamashita's reports [18]. They had confirmed that serum concentrations of HE4 were a good diagnostic marker of lung cancer (AUC: 0.825, cut-off point: 50.3pmol/l). The corresponding sensitivity and specificity were 74.5 % and 81%, respectively. However, Iwahori et al. had showed that AUC for serum HE4 was 0.988 and the sensitivity was 89.8%, specificity was 100% in their study [19]. The diagnostic effect

of lung cancer looks like much better than our study. In our opinion, this difference may be interpreted by the followings: 1) different methods were used to the detection of serum HE4 concentrations (electrochemiluminescence assays in our study, Enzyme Immunometric assay by Yamashita et al. and home-made ELISAs by Iwahori et al.); 2) patients enrolled in these studies were at different clinical stage. In our study, only patients at stage I and II and local limited disease were enrolled, but patients with advanced disease were enrolled in other studies.

It is known that most biomarkers in pulmonary cancer are related to histological type. CEA was reported elevating more obviously in adenocarcinoma than in other types [20]. Cyfra21-1 was more frequently increased in squamous lung carcinoma [21]. Otherwise, NSE and proGRP were recommended as specific biomarkers of SCLC [22]. Unfortunately, in lung cancer at early stage, CEA, Cyfra21-1 and NSE were elevated in less than 20% of all patients. Serum HE4 elevated in 43.5% adenocarcinoma and 57.1% squamous early stage lung cancer. But in local limited SCLC, both proGRP and NSE elevated more obviously than HE4. Consequently, we hypothesized that combination of HE4 with proGRP and NSE might be very useful in verifying the histopathological types. We selected the best biomarkers distinguishing NSCLC from SCLC by Cox regression. It was found that proGRP alone could distinguish NSCLC from SCLC in 90.2% cases correctly, and the combination of proGRP, NSE and HE4 could improve the accuracy rate to 93.8%. The results provided a tool to predict the histological type in those hard to get tissue for biopsying.

Researchers in Japan reported that HE4 expression in serum was associated with progression of lung adenocarcinoma and higher HE4 concentration indicated worse outcome

[23]. Ximing Wang and their colleagues evaluated the expression of HE4 in SCLC, and thought HE4 was the best biomarker when distinguishing SCLC and healthy people [24]. The rational explain was that they did not detect proGRP which was proved the most sensitive biomarker of SCLC up to now.

According to previous reports, the concentration of HE4 was increased with the growth of the age and smoking index[25, 26]. Our study found the similar relationship between serum HE4 and age, gender and smoking index (Data not shown).

In summary, our study proved that serum HE4 was a better tumor biomarker than others in diagnosing lung cancer at early stage. It occupied the highest sensitivity in AC and SC. The combination of HE4 with proGRP and NSE provides an efficient tool to differential diagnosis NSCLC from SCLC.

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References

- [1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA: a cancer journal for clinicians* 2011; 61:69-90.
- [2] Edwards BK, Noone AM, Mariotto AB, et al. Annual Report to the Nation on the status of cancer, 1975-2010, featuring prevalence of comorbidity and impact on survival among persons with lung, colorectal, breast, or prostate cancer. *Cancer* 2014; 120:1290-1314.
- [3] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA: a cancer journal for clinicians* 2009; 59:225-249.
- [4] Henschke CI, Yankelevitz DF. CT screening for lung cancer: update 2007. *The oncologist* 2008; 13:65-78.
- [5] Diamandis EP, Hoffman BR, Sturgeon CM. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for the Use of Tumor Markers. *Clinical chemistry* 2008; 54:1935-1939.
- [6] Kulpa J, Wojcik E, Reinfuss M, Kolodziejwski L. Carcinoembryonic antigen, squamous cell carcinoma antigen, CYFRA 21-1, and neuron-specific enolase in squamous cell lung cancer patients. *Clinical chemistry* 2002; 48:1931-1937.
- [7] Escudero JM, Auge JM, Filella X, Torne A, Pahisa J, Molina R. Comparison of serum human epididymis protein 4 with cancer antigen 125 as a tumor marker in patients with malignant and nonmalignant diseases. *Clinical chemistry* 2011; 57:1534-1544.
- [8] Karlsen NS, Karlsen MA, Hogdall CK, Hogdall EV. HE4 tissue expression and serum HE4 levels in healthy individuals and patients with benign or malignant tumors: a systematic review. *Cancer epidemiology, biomarkers & prevention : a publication of the American*

Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 2014; 23:2285-2295.

[9] Wang K, Gan L, Jeffery E, et al. Monitoring gene expression profile changes in ovarian carcinomas using cDNA microarray. *Gene* 1999; 229:101-108.

[10] Shridhar V, Lee J, Pandita A, et al. Genetic analysis of early- versus late-stage ovarian tumors. *Cancer research* 2001; 61:5895-5904.

[11] Hellstrom I, Raycraft J, Hayden-Ledbetter M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. *Cancer research* 2003; 63:3695-3700.

[12] Tokuishi K, Yamashita S, Ohbo K, Kawahara K. Splice variant HE4-V3 expression is associated with favorable prognosis in pulmonary adenocarcinoma. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2012; 33:103-109.

[13] Hertlein L, Stieber P, Kirschenhofer A, et al. Human epididymis protein 4 (HE4) in benign and malignant diseases. *Clinical chemistry and laboratory medicine : CCLM / FESCC* 2012; 50:2181-2188.

[14] Galgano MT, Hampton GM, Frierson HF, Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2006; 19:847-853.

[15] Rami-Porta R, Wittekind C, Goldstraw P, International Association for the Study of Lung Cancer Staging C. Complete resection in lung cancer surgery: proposed definition. *Lung cancer* 2005; 49:25-33.

[16] Zelen M. Keynote address on biostatistics and data retrieval. *Cancer chemotherapy*

reports Part 3 1973; 4:31-42.

[17]Bingle L, Cross SS, High AS, et al. WFDC2 (HE4): a potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. *Respiratory research* 2006; 7:61.

[18]Yamashita S, Tokuishi K, Moroga T, et al. Serum level of HE4 is closely associated with pulmonary adenocarcinoma progression. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2012; 33:2365-2370.

[19]Iwahori K, Suzuki H, Kishi Y, et al. Serum HE4 as a diagnostic and prognostic marker for lung cancer. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 2012; 33:1141-1149.

[20]Sakao Y, Tomimitsu S, Takeda Y, Natsuaki M, Itoh T. Carcinoembryonic antigen as a predictive factor for postoperative tumor relapse in early-stage lung adenocarcinoma. *European journal of cardio-thoracic surgery : official journal of the European Association for Cardio-thoracic Surgery* 2004; 25:520-522.

[21]Liu L, Liu B, Zhu LL, Zhang W, Li Y. Clinical significance of CYFRA21-1, Scc-Ag and telomerase activity in serum and pleural effusion of patients with squamous-cell lung cancer. *Bioanalysis* 2012; 4:2367-2374.

[22]Miyake Y, Kodama T, Yamaguchi K. Pro-gastrin-releasing peptide(31-98) is a specific tumor marker in patients with small cell lung carcinoma. *Cancer research* 1994; 54:2136-2140.

[23]Yamashita S, Tokuishi K, Hashimoto T, et al. Prognostic significance of HE4 expression in pulmonary adenocarcinoma. *Tumour biology : the journal of the International Society for*

Oncodevelopmental Biology and Medicine 2011; 32:265-271.

[24] Wang X, Fan Y, Wang J, Wang H, Liu W. Evaluating the expression and diagnostic value of human epididymis protein 4 (HE4) in small cell lung cancer. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 2014; 35:6847-6853.

[25] Bolstad N, Oijordsbakken M, Nustad K, Bjerner J. Human epididymis protein 4 reference limits and natural variation in a Nordic reference population. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 2012; 33:141-148.

[26] Nagy B, Jr., Bhattoa HP, Steiber Z, et al. Serum human epididymis protein 4 (HE4) as a tumor marker in men with lung cancer. Clinical chemistry and laboratory medicine : CCLM / FESCC 2014; 52:1639-1648.

Fig. legend

Fig. 1 Distribution of serum HE4 and other tumor markers in NSCLC, SCLC, lung cancers and healthy controls. HC: Healthy controls; LC: Lung cancer patients

Fig. 2 ROC curve analysis of serum HE4 and other tumor markers. A: in all participants. The combination of all biomarkers were expressed by equation: $P = 0.213 \times \text{NSE} + 0.570 \times \text{Cyfra21-1} + 0.099 \times \text{HE4} + 0.011 \times \text{proGRP} - 9.343$. B: in AC patients and healthy control. C: in SC patients and healthy controls. D: in SCLC patients and healthy controls

A **B** **C** **D**

Table 1 Characteristics of study participants

Diagnosis	n	Sex	
		Male	Female
Healthy Control	50	31	19
Lung Cancer	112	72	40
NSCLC	81	52	29
Stage I	62	37	25
Stage II	19	15	4
SC	35	31	4
AC	46	21	25
SCLC(limited stage)	31	20	11

Table 2 The median value of serum HE4 and other tumor markers in different groups

Diagnosis	n	CEA(<u>ug/L</u>)		NSE(<u>ug/L</u>)		Cyfra21-1(<u>ug/L</u>)		HE4(<u>pmol/L</u>)		proGRP(<u>ng/L</u>)	
		median(range)		median(range)		median(range)		median(range)		median(range)	
Healthy Control [※]	50	1.99	(0.54-7.69)	11.29	(1.78-17.09)	1.91	(0.92-4.15)	48.50	(32.68-79.63)	42.52	(27.94-80.68)
Lung Cancer	112	2.96	(0.33-89.51)	12.27	(6.82-91.58)	2.67	(0.78-17.67)	64.72	(29.89-233.10)	42.74	(15.47-1537.00)
NSCLC [▲] _a	81	3.00	(0.33-89.51)	11.65	(6.82-24.70)	2.86	(0.78-17.67)	66.05	(40.17-233.10)	39.31	(15.47-88.97)
SC ^b	35	2.95	(0.59-13.27)	12.15	(7.99-24.70)	2.94	(1.25-17.67)	71.09	(40.17-233.10)	37.42	(15.47-85.85)
AC	46	3.40	(0.33-89.51)	11.48	(6.82-16.97)	2.67	(0.78-6.46)	64.69	(42.80-172.70)	39.56	(16.22-88.97)
SCLC [△]	31	1.96	(0.68-86.88)	15.11	(7.00-91.58)	2.17	(1.20-8.19)	57.43	(29.89-106.80)	159.50	(16.62-1537.00)

[※] comparison between lung cancer and controls. (CEA:*P*=0.046; NSE:*P*=0.001; Cyfra21-1:*P*<0.0001; HE4:*P*<0.0001; proGRP: *P*=0.934)

[★] comparison between NSCLC patients and healthy controls. (CEA:*P*=0.009; NSE:*P*=0.042; Cyfra21-1:*P*<0.0001; HE4:*P*<0.0001; proGRP:*P*=0.014)

[△] comparison between SCLC patients and healthy controls. (CEA:*P*=0.977; NSE:*P*<0.0001; Cyfra21-1:*P*=0.128; HE4: *P*=0.002; proGRP: *P*<0.0001)

^a comparison between NSCLC and SCLC.(CEA:*P*=0.053; NSE:*P*<0.0001; Cyfra21-1:*P*=0.022; HE4: *P*=0.010; proGRP: *P*<0.0001)

^b comparison between SC and AC. (CEA:*P*=0.571; NSE:*P*=0.157; Cyfra21-1:*P*=0.026; HE4: *P*=0.312; proGRP: *P*=0.249)

Table 3 Sensitivity and specificity of biomarkers alone or combined in diagnosis of lung cancer.

	AUC	95%CI	cut-off	sensitivity	specificity	Youden's index
A-Lung cancer						
HE4	0.821	0.754-0.887	66.8	43.8%	95.0%	0.388
CEA	0.598	0.507-0.689	5.89	15.2%	95.0%	0.102
NSE	0.660	0.570-0.750	16.5	17.0%	95.0%	0.120
Cyfra21-1	0.699	0.613-0.785	3.79	17.9%	95.0%	0.129
ProGRP	0.504	0.416-0.592	73.5	23.2%	95.0%	0.182
proGRP+HE4				48.2%	96.0%	0.442
combination of biomarkers [※]	0.844	0.832-0.937	2.1	51.8%	95.0%	0.468
B-Adenocarcinoma						
HE4	0.868	0.797-0.939	66.8	43.5%	95.0%	0.385
CEA	0.646	0.534-0.758	5.89	19.6%	95.0%	0.146
NSE	0.571	0.455-0.686	16.5	4.3%	95.0%	-
Cyfra21-1	0.683	0.576-0.790	3.79	13.0%	95.0%	0.080
ProGRP	0.406	0.292-0.521	73.5	4.3%	95.0%	-
HE4+CEA				52.2%	92.0%	0.442
C-Squamous carcinoma						
HE4	0.863	0.779-0.948	66.8	57.1%	95.0%	0.521
CEA	0.625	0.504-0.745	5.89	11.4%	95.0%	0.064
NSE	0.652	0.536-0.769	16.5	11.4%	95.0%	0.064
Cyfra21-1	0.807	0.715-0.899	3.79	31.4%	95.0%	0.264
ProGRP	0.328	0.211-0.445	73.5	2.9%	95.0%	-
HE4+Cyfra21-1				62.9%	94.0%	0.619
D-Small-cell lung carcinoma						
HE4	0.703	0.579-0.826	66.8	29.0%	95.0%	0.240
CEA	0.498	0.364-0.632	5.89	12.9%	95.0%	0.079
NSE	0.802	0.699-0.904	16.5	41.9%	95.0%	0.369
Cyfra21-1	0.601	0.474-0.728	3.79	9.7%	95.0%	0.047
ProGRP	0.848	0.745-0.952	73.5	74.2%	95.0%	0.692
NSE+proGRP				80.6%	92.0%	0.726

* Equation from Cox regression: $P=0.213 \times \text{NSE} + 0.570 \times \text{Cyfra21-1} + 0.099 \times \text{HE4} + 0.011 \times \text{proGRP} - 9.343$

Table 4 Application of biomarkers in distinguishing NSCLC from SCLC

			NSCLC	SCLC	Percentage Correct
Step1	proGRP	NSCLC	80	1	98.8
		SCLC	10	21	67.7
		Overall Percentage			90.2
Step2	proGRP+HE4	NSCLC	80	1	98.8
		SCLC	9	22	71.0
		Overall Percentage			91.1
Step3	proGRP+HE4+NSE	NSCLC	81	1	98.8
		SCLC	6	25	80.6
		Overall Percentage			93.8