Int J Clin Exp Pathol 2016;9(6):5970-5979
www.ijcep.com /ISSN:1936-2625/IJCEP0025429

Original Article

Prognostic value of neuroendocrine markers for predicting survival in patients with small cell lung cancer

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Received February 2, 2016; Accepted April 26, 2016; Epub June 1, 2016; Published June 15, 2016

Abstract: Patients with small cell lung carcinoma (SCLC) present neuroendocrine (NE) properties and chromogranin A (CGA), synaptophysin (Syn), and neural cell adhesion molecule 1 (NCAM1) are known as NE diagnostic markers, while the predictive value of the NE differentiation in SCLC remains uncertain. The aim of this retrospective study was to evaluate the prognostic significance of NE markers in tissue level in SCLC. A total of 192 patients were enrolled in the study, 136 (70.8%) patients received chemotherapy, and 10 (5.2%) patients were underwent lobectomy resection. The expression levels of the NE markers were performed using immunohistochemistry (IHC). Our data showed the positive expression rates of CGA, Syn, and NCAM1 were 112 (58.3%), 160 (83.3%), 166 (86.5%), respectively. There were significant associations between CGA, NE markers and disease stage, lymph node metastasis in SCLC (P<0.05). A multivariate analysis was performed using Cox regression model, the results showed that disease stage (P<0.001), regional lymph node metastasis (P<0.001), CGA expression (P=0.019), NE differentiation (P=0.033), chemotherapy (P=0.001), and surgery (P=0.001) were independent factors associated with overall survival. Kaplan-Meier and log-rank test demonstrated that patients with low expression of CGA (Log rank P=0.015) and low level of NE markers (Log rank P=0.028) proved to have significant longer survival compared to those with the opposite status of each variable. In conclusion, low CGA level or low NE markers by IHC in SCLC are both prognostic determinants. Further studies are needed to elucidate the function of particular SCLC subtype in cell lines.

Keywords: Small-cell lung carcinoma, CGA, neuroendocrine differentiation, immunohistochemistry

Introduction

Lung cancer remains one of the most common worldwide malignancies. More than 85% of lung cancer patients are diagnosed as non-small cell lung cancer (NSCLC), while the other 15% of lung cancer patients are classified as small cell lung cancer (SCLC) [1]. SCLC is one of the most aggressive malignant neuroendocrine (NE) tumors consisting of small cells that derive from the lung [2, 3]. All the patients show histological features of NE morphology. It is believed that SCLC progresses more rapidly with poor survival compared to non-SCLC. Most of the cases have early metastases at the time of diagnosis, resulting in combination chemotherapy treatment but not surgery. Even SCLC patients are sensitive to the chemotherapy, most of the tumor could recurrence in a short time [4]. Thus, in order to optimize treatment and improve prognosis of patients with SCLC, it is necessary to identify a novel predictor of efficacy and outcome for SCLC.

Chromogranin A (CGA) is a 49 kDa heat stable acidic glycoprotein whose coded gene is locat-

ed on chromosome 14. It is commonly expressed in the secretory granules of many normal and malignant neuroendocrine cells [5, 6]. Besides, some tumors including breast, non-small cell lung cancer, gastric and colorectal, prostate cancer can present focal positive expression of CGA. To our knowledge, CGA is well described as a tissue marker for SCLC diagnosis. In addition, CGA expression level was considered as predictors of small cell carcinoma of the cervix, gastroenteropancreatic neuroendocrine neoplasm, prostate cancer [7-9]. However, although previous studies have examined the prognostic capability of serum level of CGA by ELISA in SCLC, discrepancy is generated by different determination of cutoff values, and inconsistent data is obtained [7-12]. In our present study, we evaluated the expression level of CGA in tissue by immunohistochemistry (IHC).

Synaptophysin (Syn) was one of the first synaptic proteins which was identified more than 4 decades ago, this gene encodes an integral membrane protein of small synaptic vesicles which is ubiquitously expressed in synapses throughout the mammalian brain [13]. Syn is known to bind cholesterol, besides, Syn can direct target vesicle-associated membrane protein 2 (synaptobrevin) to intracellular compartments. Mutations in Syn gene which is involved in neuroscience and synaptic vesicle cycle are associated with mental retardation, X-linked 96 and Syn-related x-linked mental retardation. Among its related pathways are. Syn is believed as a reliable neuroendocrine tissue marker and was commonly used in the diagnosis of neuroblastoma, pheochromocytoma, pituitary adenoma, thyroid adenoma, small cell carcinoma of the prostate and SCLC, whether Syn can be treated as a prognosis marker for SCLC remains to be explored [14, 15].

Neural Cell Adhesion Molecule 1 (NCAM1, also known as CD56) is a cell adhesion protein which belongs to immunoglobulin superfamily [16, 17]. It plays a key role in cell-to-cell interactions as well as cell-matrix interactions during development of embryos and nervous system [18, 19]. Many researchers have paid attention to its role in retinal blastoma, medulloblastoma, astrocytoma, neuroblastoma, gallbladder carcinoma, colorectal cancer, papillary thyroid carcinoma and acute myeloid leukemia [20-22]. Besides, NCAM1 is also implicated in the expansion of T cells and dendritic cells which play a key role in immune surveillance. It was usually used as a marker for the diagnosis of NK/T cell lymphoma, myeloma and SCLC. While the predictive value of NCAM1 in SCLC remains to be elucidated.

In this retrospective study, we investigate the pathogenetic and prognostic role of the IHC expression of NE markers including CGA, Syn and NCAM1 in SCLC. It is hoped that this study will give some information for the guidance of treatment and prognosis for SCLC.

Materials and methods

Patients

A total of 192 patients with confirmed SCLC in the Shaanxi Province Hospital recruited into our retrospective study between January 2009 and December 2012. Histologic diagnosis of SCLC was made based on the 2004 WHO classification. Tumor specimens were obtained by surgical resection, CT-guided transthoracic lung biopsy, or bronchoscopic biopsy. Formalin-fixed and paraffin-embedded (FFPE) tissues were made from the collected specimens for diagnosis and immunohistochemical (IHC) staining. All the patients underwent normal clinical examination, besides, CT scans of the chest, abdomen and brain were performed, and a bone scan was also carried out. No patients had been diagnosed as any other cancer; no patients had received anticancer therapy prior to enrollment in the present study. Patients with inaccurate medical records were excluded. Staging was classified according to the veterans administration lung cancer group (VALG) staging system.

The patients were treated by platinum-based chemotherapy with or without surgery. The response to therapy was evaluated after two treatment cycles according to the Response Evaluation Criteria in Solid Tumors guidelines (RECIST) 1.1 criteria. The treatment response to initial treatment was classified as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Accordingly, patients who had stable disease (SD), and progression disease (PD) were considered as non-responders, while patients who had complete response (CR) and partial response (PR) were classified as responders.

Table 1. The main clinical characteristics of the patients with SCLC

Clinicopathological data	N (%)
Age	
≤60	114 (59.4%)
>60	78 (40.6%)
Gender	
Male	139 (72.4%)
Female	53 (27.6%)
Tumor size	
≤5 cm	43 (74.5%)
>5 cm	49 (25.5%)
Initial stage	
Limited stage	31 (16.1%)
Extensive stage	131 (68.2%)
Regional lymph node metastasis	
Absent	20 (10.4%)
Present	140 (72.9%)
Chemotherapy	
No	56 (29.2%)
Yes	136 (70.8%)
Surgery	
Yes	10 (5.2%)
No	182 (94.8%)
CGA	
Negative	80 (41.7%)
Positive	112 (58.3%)
Syn	
Negative	32 (16.6%)
Positive	160 (83.3%)
NCAM1	
Negative	26 (13.5%)
Positive	166 (86.5%)
NE markers	
Low	108 (56.3%)
High	84 (43.8%)
Median survival time (Months, range)	7 (2-35)

Abbreviations: CGA = Chromogranin A; Syn = Synaptophysin; NCAM1 = Neural Cell Adhesion Molecule 1.

Of the 136 patients treated by platinum-based chemotherapy, 121 (89.0%) patients were responders and 15 (11.0%) were non-responders.

The follow-up information was collected by telephone interview from the patients or their relatives. The Overall survival (OS) was counted from the date of diagnosis to the date of death or last follow-up. The detailed clinicopathological data including age, gender, lymph node metastasis, initial stage, and survival data, therapeutic strategies were summarized in **Table 1**. This study was approved by Ethics Committee of Medical College of Xi'an Jiaotong University, Xi'an, China; informed consent was not required because of the retrospective nature of the study. All participants provided consent for the sample collection and data analysis.

Immunohistochemistry

CGA, Syn and NCAM1 protein expression levels were evaluated by immunohistochemistry staining. A formalin-fixed, paraffin-embedded (FFPE) tissue block from each patient was cut into 4 μm sections for IHC staining. All sections were dewaxed in xylene and rehydrated with a graded ethanol series. In order to do antigen retrieval, the sections were placed in an autoclave for 10 min in 10 mM citrate buffer (pH 6.0). Then, 3% hydrogen peroxide was used for 10 min to block endogenous peroxidase activity. Sequentially, slides were incubated with the CGA primary antibody (Cell Signaling Technology, MA, USA), Syn primary antibody (Proteintech Group, Inc, Wuhan, China) and NCAM1 primary antibody (Cell Signaling Technology, MA, USA) overnight at 4°C, SCLC was used as positive control, PBS was used instead of primary antibody as negative control. Next, HRP-conjugated secondary antibody (Maixin biological, Fuzhou, China) was applied for 15 min at room temperature, 3,3'-diaminobenzidine (DAB) was used as a chromogen. Finally, all the slides were washed in water, counterstained with haematoxylin, dehydrated with ethanol, cleaned with xylene and mounted by cover slips. Immunostained slides were interviewed by two independent pathologists who were blinded to the clinicopathological data. Staining was considered positive if tumor cells presented focal, patchy, or diffuse staining intracellularly as previously described [23, 24]. We defined that patients with any one of positive CGA, Syn or NCAM1 as the NE+ subgroup, patients with any two of positive CGA, Syn or NCAM1 as the NE2+ subgroup, patients with total positive CGA, Syn and NCAM1 as NE3+ subgroup, while patients with total negative CGA, Syn or NCAM1 was defined as NE- subgroup. Accordingly, patients with NE- and NE+ were divided into NE low subgroup, and patients with NE2+ and NE3+ were divided into NE high subgroup.

Table 2. The main clinical characteristics of patients according to CGA, Syn and NCAM1 expression levels

Clinicopathological data	N (%)	CGA		χ^2	P	Syn		χ^2	P	NCAM1		χ^2	P	NE markers		χ^2	P
		Negative	Positive			Negative	Positive			Negative	Positive			Low	High		
Age																	
≤60	116 (60.4%)	50 (62.5%)	66 (58.9%)	0.249	0.618	18 (56.3%)	98 (61.3%)	0.279	0.598	17 (65.4%)	99 (59.6%)	0.310	0.577	68 (63.0%)	48 (57.1%)	0.669	0.413
>60	76 (39.6%)	30 (37.5%)	46 (41.1%)			14 (43.8%)	62 (38.8%)			9 (34.6%)	67 (40.4%)			40 (37.0%)	36 (42.9%)		
Male	142 (74.0%)	60 (75.0%)	82 (73.2%)	0.077	0.781	27 (84.4%)	115 (71.9%)	2.163	0.141	19 (73.1%)	123 (74.1%)	0.012	0.912	81 (75.0%)	61 (72.6%)	0.139	0.709
Female	50 (26.0%)	20 (25.0%)	30 (26.8%)			5 (15.6%)	45 (28.1%)			7 (26.9%)	43 (25.9%)			27 (25.0%)	23 (27.4%)		
Tumor size																	
≤5 cm	90 (46.9%)	32 (40.0%0	58 (51.8%)	2.603	0.107	17 (53.1%)	73 (45.6%)	0.602	0.483	14 (53.8%)	76 (45.8%)	0.587	0.444	49 (45.4%)	41 (48.8%)	0.224	0.636
>5 cm	102 (53.1%)	48 (60.0%)	54 (48.2%)			15 (46.9%)	87 (54.4%)			12 (46.2%)	90 (54.2%)			59 (54.6%)	43 (51.1%)		
Initial stage																	
Limited stage	38 (19.8%)	21 (26.3%)	17 (15.2%)	3.603	0.058	8 (25.0%)	30 (18.8%)	0.656	0.418	8 (30.8%)	30 (18.1%)	2.283	0.131	28 (25.9%)	11 (13.1%)	4.666	0.031
Extensive stage	154 (80.2%)	59 (73.8%)	95 (84.8%)			24 (75.0%)	130 (81.3%)			18 (69.2%)	136 (81.9%)			80 (74.1%)	73 (86.9%)		
Regional lymph node metastasis																	
Absent	33 (17.2%)	19 (23.8%)	14 (12.5%)	4.150	0.042	7 (21.9%)	26 (16.3%)	0.593	0.441	7 (26.9%)	26 (15.7%)	2.003	0.157	23 (21.3%)	10 (11.9%)	2.928	0.087
Present	159 (82.8%)	61 (76.3%)	98 (87.5%)			25 (78.1%)	134 (83.8%)			19 (73.1%)	140 (84.3%)			85 (78.7%)	74 (88.1%)		
Chemotherapy																	
No	39 (20.3%)	22 (27.5%)	21 (18.8%)	2.056	0.152	6 (18.8%)	33 (20.6%)	0.058	0.810	3 (11.5%)	40 (24.1%)	2.040	0.116	27 (25.0%)	16 (19.0%)	0.962	0.326
Yes	153 (79.7%)	58 (72.5%)	91 (81.3%)			26 (81.3%)	127 (79.4%)			23 (88.5%)	126 (75.9%)			81 (75.0%)	68 (81.0%)		
Surgery																	
No	179 (93.2%)	75 (93.8%)	104 (92.9%)	0.059	0.808	30 (93.8%)	149 (93.1%)	0.017	0.898	22 (84.6%)	157 (94.6%)	3.535	0.081	101 (93.5%)	78 (92.9%)	0.030	0.856
Yes	13 (6.8%)	5 (6.3%)	8 (7.1%)			2 (6.3%)	11 (6.9%)			4 (15.4%)	9 (5.4%)			7 (6.5%)	6 (7.1%)		

Abbreviations: CGA = Chromogranin A; Syn = Synaptophysin; NCAM1 = Neural Cell Adhesion Molecule 1.

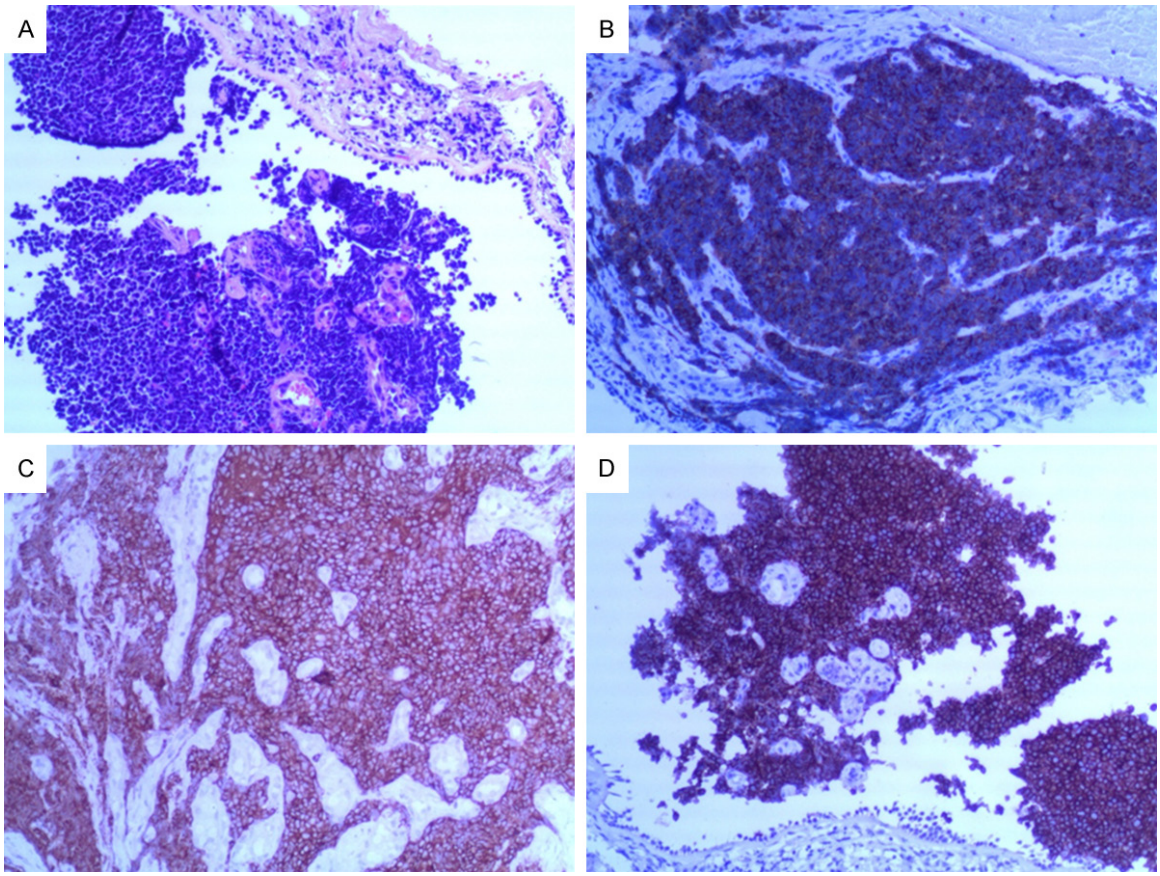

Figure 1. HE staining of SCLC as control (A), positive immunostaining of SCLC tumors for chromogranin A (B, strong), synaptophysin (C, strong), neural cell adhesion molecule (D, strong).

Statistical analysis

Data were presented as mean ± standard deviation (SD) for continuous variables, and as numbers of subjects (percentage) for categorical variables. The relationships between the continuous variables and categorical variables were assessed by student t-test and chi-square test or Fisher's exact test. The relationships between CGA, Syn and NCAM1 expression and clinicopathologic characteristics and response to treatment were evaluated by Pearson's chi-squared test and the Mann-Whitney U-test. Multivariate analysis of the prognostic factors was conducted with Cox multivariable regression models integrating clinicopathological factors to adjust for potential confounders. Survival time was counted and patients who were survived at the last contact were censored. The impacts of parameters on survival were analyzed by the Kaplan-Meier method, and differences were compared by the log-rank test between subgroups. In all statistical analyses, significance was considered as p values (two sides) ≤0.05. All data were performed using SPSS Statistics version 17.0 (SPSS, Chicago, IL, USA).

Results

Clinical characteristics and outcome of SCLC patients

The clinical characteristics of the 192 enrolled patients with SCLC (139 males and 53 females) in the present study are summarized in **Table 1**. There were 78 (40.6%) Patients with the age older than 60 years and 114 (59.4%) patients with the age younger than 60 years. 131 (68.2%) were extended disease and 31 (16.1%) were limited disease. Lymph nodal metastasis was present in 140 (72.9%) cases. 136 (70.8%) cases received at least six cycles of platinum-based chemotherapy with etoposide, and 10 (5.2%) patients underwent surgical resection with lobectomy or pneumonectomy. The data of radiotherapy was not collected. These clinical characteristics are summarized in **Table 1**.

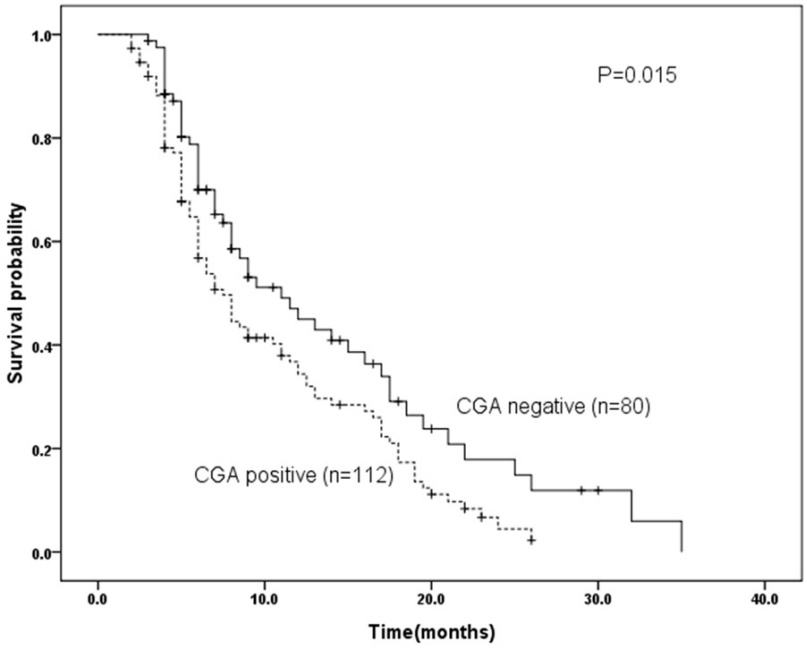

Figure 2. Kaplan-Meier curves for overall survival according to CGA expression in patients with SCLC.

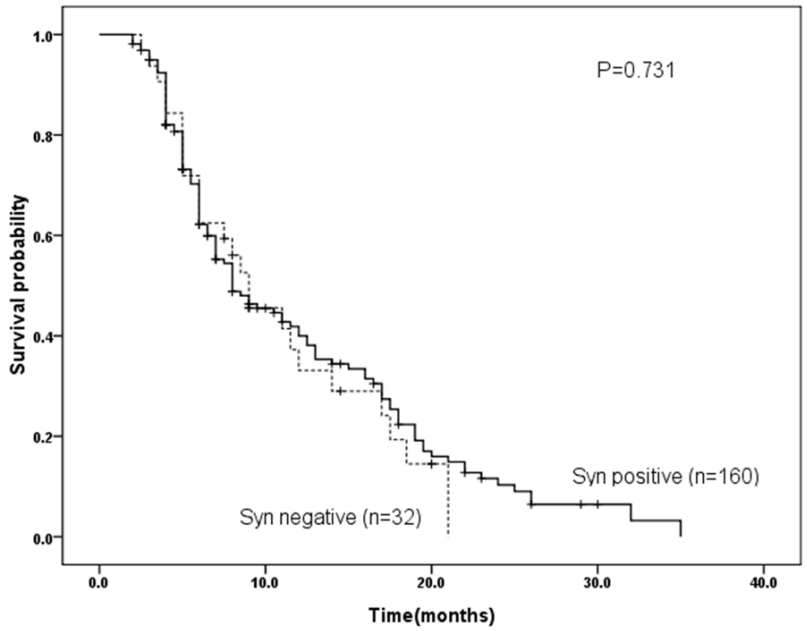

Figure 3. Kaplan-Meier curves for overall survival according to Syn expression in patients with SCLC.

Correlation between CGA, Syn and NCAM1 expression and clinical characteristics

In order to assess the clinicopathological and prognostic roles of the expression levels of three proteins, IHC analysis in paraffin-embedded SCLC sections were performed. Clinicopathological characteristics of SCLC are classified according to CGA, Syn and NCAM1 expression status, respectively (**Table 2**). Of the 192 tumors, 112 (58.3%) were positive for CGA, 160 (83.3%) were positive for Syn, 166 (86.5%) were positive for NCAM1 (**Figure 1**). We also analyzed the association between the three protein expression and clinical characteristics (**Table 2**).

No significant association was observed between NCAM1 or Syn expression and clinical characteristics including age, gender, tumor size, initial stage, regional lymph node metastasis, chemotherapy and surgery (P>0.05). Of note, there was more patients with lymph node metastasis in CGA positive group compare to those in CGA negative group (P= 0.042). Besides, CGA had a trend higher expression in SCLCs with extended-stage compare to those with limited-stage patients (P=0.058). Furthermore, the patients with extended-stage tended to have higher NE differentiation than those with limited-stage (P=0.031).

Correlation between CGA, Syn and NCAM1 expression and patient survival

All patients were followed up until December, 2012, during follow-up, 50 (26.1%) patients were survived, while 142 (74.0%) died from disease progression. The median overall survival time was 7 months (range: 2-35 months). Kaplan-Meier survival analysis showed that the

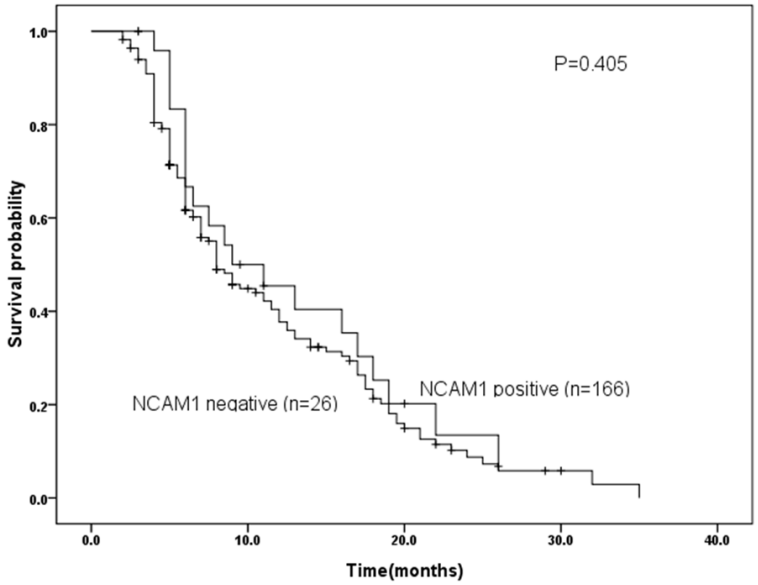

Figure 4. Kaplan-Meier curves for overall survival according to NCAM1 expression in patients with SCLC.

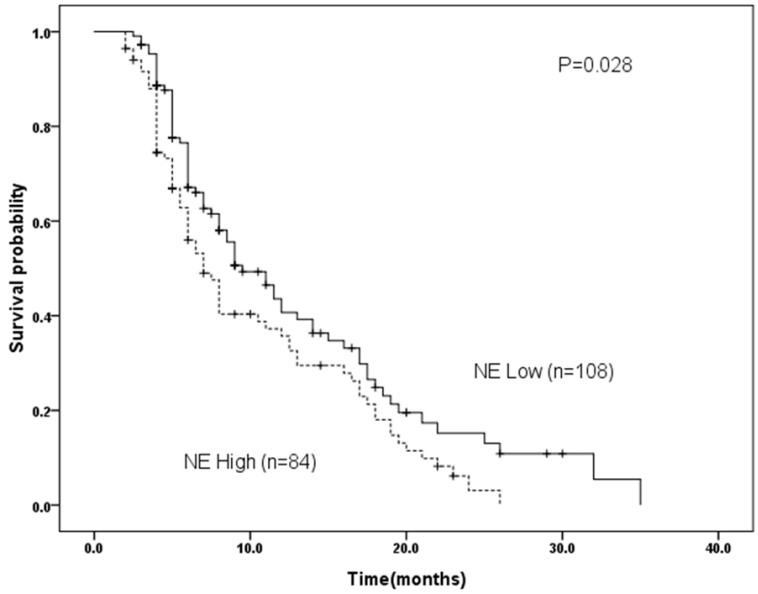

Figure 5. Kaplan-Meier curves for overall survival according to NE markers expression in patients with SCLC.

overall survival time were significantly shorter in the CGA overexpression subgroup compare with CGA under expression (Log rank P=0.015) (**Figure 2**). However, no significant difference existed between the two groups with higher or lower expression level of Syn (Log-rank P=0.731, **Table 2**; **Figure 3**) and NCAM1 (Log-rank P=0.405, **Table 2**; **Figure 4**). Interestingly, as described in the materials and methods section, when we combined the CGA, Syn and NCAM1 together, the survival time was significantly shorter in NE high subgroup than in NE low subgroup (Log-rank P= 0.028) (**Figure 5**).

The predictive effect of clinicopathologic factors is shown in **Table 3**. A multivariate analysis was performed using Cox regression model, the results showed that disease stage (P< 0.001), lymph node metastasis (P<0.001), CGA expression (P=0.019), NE differentiation (P=0.033), chemotherapy (P= 0.001), and surgery (P=0.001) were independent factors associated with overall survival (**Table 3**).

Discussion

During the last decade, some accomplishment has been made in the treatment of SCLC. The survival time has been significantly improved for patients with limited or extensive-stage disease. All these progression is believed to result from improvement of systematic and standard treatment therapy and accurate selection of patients who will be able to benefit from intensive therapy. Currently, some new predictive markers are needed for subclassification of patients with homogeneous prognosis for the availability of novel treatment strategies. One example is the application of epidermal growth factor receptor (EGFR) inhibitor on a subgroup of pulmonary adenocarcinomas [25, 26].

Tumor markers are potential prognostic determining factors of SCLC. Patients with SCLC present neuroendocrine properties, Syn, CGA and NCAM1 are putative serum tumor markers

Table 3. Cox regression analysis of the factors affecting the survival of the patients

Variables	P
Age	0.530
Gender	0.383
Tumor size	<0.001
Initial stage	<0.001
Regional lymph node metastasis	<0.001
Chemotherapy	0.001
Surgery	0.001
CGA	0.019
Syn	0.739
NCAM1	0.420
NE markers	0.033

Abbreviations: CGA = Chromogranin A; Syn = Synaptophysin; NCAM1 = Neural Cell Adhesion Molecule 1.

which are routinely used for IHC diagnosis of neuroendocrine tumors including SCLC. Syn is an integral membrane protein of presynaptic vesicles that is ubiquitously present in synapses. It can direct target vesicle-associated membrane protein 2 (synaptobrevin) to intracellular compartments. NCAM1 is a membrane sialoglycoprotein that can mediate cell-cell adhesion through homophilic binding to NCAM on another cell. Chromogranin A is a major component of adrenal medullary catecholamine storage vesicles and it is released with epinephrine and norepinephrine during exocytosis. It is ubiquitously presents in neuroendocrine tumor cells and it is a specific marker for diagnosis of SCLC.

During the last decade, some studies have focused on the diagnostic and predictive value of NE markers including Syn, CGA and NCAM1 in serum levels in SCLC. However, the discrepancy is generated by different determination of cutoff values, and inconsistent data is obtained. In our present study, we investigated the survival association between clinicopathological parameters and the three NE markers by IHC and SCLC. We found out that patients with extensive-stage have a shorter survival and disease stage was a significant prognostic determinant in the multivariate Cox model, while smoking status and lymphoma node metastasis were excluded from the Cox model after confounders adjusting. Interestingly, CGA was associated with disease stage, and there was a significant correlation between CGA expression level and SCLC survival. In other words, survival is significantly worse for patients with overexpression CGA and CGA is a significant predictive marker also in multivariable analysis. This data, to some extent, is consistent with the other results in serum level which showed that elevated level of CGA is associated with poor survival in patients with SCLC [10, 11]. To our knowledge, although some reports explored the prognosis of CGA expression level with non-SCLC [24, 27], this is the first study which indicates that CGA expression level by IHC (other than serum level) could be treated as a predictive marker for SCLC. Of note, the positive rate of CGA is 58.3%, more prognostic markers should be considered together in order to have more accurate prediction. No association was observed between SCLC survival and NCAM or Syn. This may be due to the small size of the selected population, since the negative rates of NCAM and Syn were 13.5% and 16.6%, respectively.

Recently, it was demonstrated by one report that SCLC patients who were treated only by lobectomy resection with low NE markers have good prognosis [23]. However, the population is relatively small, larger sample size study are needed to verify the results. To strengthen validity, the present study analyzed a large number of population and focused on characterizing the SCLC subgroup by hypothesizing that low expression level of NE markers (CGA, Syn and NCAM1) of tumor cells might have better prognosis compare to high expression of NE markers. 192 patients were enrolled in our present study, population were divided into two groups including NE low subgroup (including NE- and NE+) and NE high subgroup (including NE2+ and NE3+) based on the expression levels of the three NE markers. Our study is, to some extent, in accordance with the previous report, the patients in NE low subgroup have a better prognosis compare to those in NE high subgroup [23]. Besides, NE subgroup was proved to be an independent factor which influence the outcome of SCLC (P=0.033). Of note, the definition of NE differentiation is similar between the previous study and our cohort.

There are some explanations need to be clarify. Firstly, although the expression pattern of NE differentiation would be more precise with a larger size of cohort, this study demonstrates

some important aspects for carcinogenesis, subclassification and potential therapeutic options. Secondly, identification of potential tissue markers may be favorable for the decision of therapeutic regimens. In this regard, CGA could be treated as a target in NE differentiated tumors, while for those with CGA negative, NE marker including CGA, Syn and NCAM1 is a surrogate option. Thirdly, chemosensitivity and radiosensitivity is critical for SCLC treatment. Unfortunately, radiotherapy was excluded in this study, since treatment was performed by radioactive seed implantation or radiotherapy based on the individual clinical option. Besides, adjuvant chemotherapy was not correlated with CGA expression and NE differentiation in our cohort. This may be partly resulting from the small population size and few patients who were treated by chemotherapy. Further study accumulating more cases is needed to explore the relationship between NE markers and chemotherapy response. Finally, NE differentiation of SCLC have been reported in some studies, however, till now, there is no unambiguous "definition" or "gold standard" of NE differentiation. Investigation of the NE markers by IHC methods are frequently used in clinical pathology.

In conclusion, overexpression of CGA by IHC methods is a poor prognostic determinant of outcome of SCLC patients. Besides, NE-low subgroup (including NE- and NE+) had a much better survival compare to NE-high subgroup (including NE2+ and NE3+). CGA could be treated as a surrogate when NE markers including CGA, Syn and NCAM1 are not available in routine practice. Our present study merits further investigation of NE related markers in future SCLC treatment trials.

Acknowledgements

This work was partly supported by Postdoctoral Science Foundation of Shaanxi Province (2014). We would like to thank all the participants and staff of Shaan'Xi Tumor Hospital, for their valuable contributions on collecting specimens and sorting out clinical data.

Disclosure of conflict of interest

None.

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References

[1] Siegel R, Naishadham D and Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62: 10-29.

[2] Oser MG, Niederst MJ, Sequist LV and Engelman JA. Transformation from non-small-cell lung cancer to small-cell lung cancer: molecular drivers and cells of origin. Lancet Oncol 2015; 16: e165-172.

[3] Ren Y, Hou J, Xu A and Pan Y. Diagnostic utility of PAX2 and PAX5 in distinguishing non-small cell lung cancer from small cell lung cancer. Int J Clin Exp Pathol 2015; 8: 14709-14716.

[4] Hartley CP, Steinmetz HB, Memoli VA and Tafe LJ. Small cell neuroendocrine carcinomas of the lung do not harbor high-risk human papillomavirus. Hum Pathol 2015; 46: 577-582.

[5] Cheng Y, Sun Z, Bai C, Yan X, Qin R, Meng C and Ying H. Serum chromogranin A levels for the diagnosis and follow-up of well-differentiated non-functioning neuroendocrine tumors. Tumour Biol 2016; 37: 2863-9.

[6] Fan S, Hao ZY, Zhang L, Chen XG, Zhou J, Zang YF, Tai S and Liang CZ. Increased chromogranin A and neuron-specific enolase in rats with chronic nonbacterial prostatitis induced by 17-beta estradiol combined with castration. Int J Clin Exp Pathol 2014; 7: 3992-3999.

[7] Liao LM, Zhang X, Ren YF, Sun XY, Di N, Zhou N, Pan RK, Ma SH and Zhou LX. Chromogranin A (CgA) as poor prognostic factor in patients with small cell carcinoma of the cervix: results of a retrospective study of 293 patients. PLoS One 2012; 7: e33674.

[8] Wang YH, Yang QC, Lin Y, Xue L, Chen MH and Chen J. Chromogranin A as a marker for diagnosis, treatment, and survival in patients with gastroenteropancreatic neuroendocrine neoplasm. Medicine (Baltimore) 2014; 93: e247.

[9] Conteduca V, Burgio SL, Menna C, Carretta E, Rossi L, Bianchi E, Masini C, Amadori D and De Giorgi U. Chromogranin A is a potential prognostic marker in prostate cancer patients treated with enzalutamide. Prostate 2014; 74: 1691-1696.

[10] Drivsholm L, Paloheimo LI and Osterlind K. Chromogranin A, a significant prognostic factor in small cell lung cancer. Br J Cancer 1999; 81: 667-671.

[11] Pujol JL, Quantin X, Jacot W, Boher JM, Grenier J and Lamy PJ. Neuroendocrine and cytokeratin serum markers as prognostic determinants of small cell lung cancer. Lung Cancer 2003; 39: 131-138.

[12] Petrovic M, Bukumiric Z, Zdravkovic V, Mitrovic S, Atkinson HD and Jurisic V. The prognostic significance of the circulating neuroendocrine markers chromogranin A, pro-gastrin-releasing peptide, and neuron-specific enolase in patients with small-cell lung cancer. Med Oncol 2014; 31: 823.

[13] Adams DJ, Arthur CP and Stowell MH. Architecture of the Synaptophysin/Synaptobrevin Complex: Structural Evidence for an Entropic Clustering Function at the Synapse. Sci Rep 2015; 5: 13659.

[14] Ather MH, Abbas F, Faruqui N, Israr M and Pervez S. Correlation of three immunohistochemically detected markers of neuroendocrine differentiation with clinical predictors of disease progression in prostate cancer. BMC Urol 2008; 8: 21.

[15] Dorff TB, Liu SV, Xiong S, Cai J, Hawes D and Pinski J. Ethnic differences in neuroendocrine expression in prostate cancer tissue. Anticancer Res 2011; 31: 3897-3901.

[16] Mehrabian M, Brethour D, Wang H, Xi Z, Rogaeva E and Schmitt-Ulms G. The Prion Protein Controls Polysialylation of Neural Cell Adhesion Molecule 1 during Cellular Morphogenesis. PLoS One 2015; 10: e0133741.

[17] Tajima S and Fukayama M. CD56 may be a more useful immunohistochemical marker than somatostatin receptor 2A for the diagnosis of phosphaturic mesenchymal tumors. Int J Clin Exp Pathol 2015; 8: 8159-8164.

[18] Markovic-Lipkovski J, Zivotic M, Muller CA, Tampe B, Cirovic S, Vjestica J, Tomanovic N, Zeisberg M and Muller GA. Variable Expression of Neural Cell Adhesion Molecule Isoforms in Renal Tissue: Possible Role in Incipient Renal Fibrosis. PLoS One 2015; 10: e0137028.

[19] Sterlacci W, Fiegl M, Hilbe W, Auberger J, Mikuz G and Tzankov A. Clinical relevance of neuroendocrine differentiation in non-small cell lung cancer assessed by immunohistochemistry: a retrospective study on 405 surgically resected cases. Virchows Arch 2009; 455: 125-132.

[20] Jung J, Son YS, Park H, Jeon SK, Lee JW, Choi SY, Kim JM, Kwon YG, Hong HJ and Min JK. The cell adhesion molecule L1 promotes gallbladder carcinoma progression in vitro and in vivo. Oncol Rep 2011; 25: 945-952.

[21] Kok-Sin T, Mokhtar NM, Ali Hassan NZ, Sagap I, Mohamed Rose I, Harun R and Jamal R. Identification of diagnostic markers in colorectal cancer via integrative epigenomics and genomics data. Oncol Rep 2015; 34: 22-32.

[22] Ceyran AB, Senol S, Simsek BC, Sagiroglu J and Aydin A. Role of cd56 and e-cadherin expression in the differential diagnosis of papillary thyroid carcinoma and suspected follicular-patterned lesions of the thyroid: the prognostic importance of e-cadherin. Int J Clin Exp Pathol 2015; 8: 3670-3680.

[23] Hamanaka W, Motoi N, Ishikawa S, Ushijima M, Inamura K, Hatano S, Uehara H, Okumura S, Nakagawa K, Nishio M, Horai T, Aburatani H, Matsuura M, Iwasaki A and Ishikawa Y. A subset of small cell lung cancer with low neuroendocrine expression and good prognosis: a comparison study of surgical and inoperable cases with biopsy. Hum Pathol 2014; 45: 1045-1056.

[24] Segawa Y, Takata S, Fujii M, Oze I, Fujiwara Y, Kato Y, Ogino A, Komori E, Sawada S, Yamashita M, Nishimura R, Teramoto N and Takashima S. Immunohistochemical detection of neuroendocrine differentiation in non-small-cell lung cancer and its clinical implications. J Cancer Res Clin Oncol 2009; 135: 1055-1059.

[25] Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M, Yoshioka H, Shibata K, Kudoh S, Shimizu E, Saito H, Toyooka S, Nakagawa K and Fukuoka M. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): an open label, randomised phase 3 trial. Lancet Oncol 2010; 11: 121-128.

[26] Li H, Wang Y, Su F, Li J and Gong P. Monitoring of cyclooxygenase-2 levels can predict EGFR mutations and the efficacy of EGFR-TKI in patients with lung adenocarcinoma. Int J Clin Exp Pathol 2015; 8: 5577-5583.

[27] Howe MC, Chapman A, Kerr K, Dougal M, Anderson H and Hasleton PS. Neuroendocrine differentiation in non-small cell lung cancer and its relation to prognosis and therapy. Histopathology 2005; 46: 195-201.