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The Significance of NSE and CEA as a Differentiation Marker for the Cellular Heterogeneity of Small Cell Lung Cancer

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Kobayashi, S., Okada, S., Hasumi, T., Sato, N. and Fujimura, S. *The Significance of NSE and CEA as a Differentiation Marker for the Cellular Heterogeneity of Small Cell Lung Cancer.* Tohoku J. Exp. Med., 1999, **189** (1), 37-49 —— Neuron-specific enolase (NSE) and carcinoembryonic antigen (CEA) levels in the culture supernatant of the 65 pulmonary carcinoma cell lines: Small cell lung cancer (SCLC) 18, large cell carcinoma 14, squamous cell carcinoma 14, adenocarcinoma 14 and adenosquamous cell carcinoma 5, were measured by a radioimmunoassay (RIA). The mean value of NSE was 30.8 ± 22.4 ng/ml and 9.2 ± 8.7 ng/ml in SCLC and non-SCLC, respectively. The mean value of CEA was 15.1 ± 20.9 ng/ml and 26.6 ± 72.3 ng/ml in SCLC and non-SCLC, respectively. A significant difference in NSE levels was obtained between SCLC cell lines and non-SCLC cell lines. In SCLC cell lines, a significant inverse proportional correlation was observed between NSE and CEA levels. The CEA production tended to be higher in cells with low levels of NSE than in those with high NSE production. With respect to correlation between marker production and growth characteristics of SCLC cells in vitro, significantly higher NSE and lower CEA levels were found in cells growing with floating colony or neurite like characteristics (classic cell type) than those in cells with epithelial or intermediate growth characteristics (variant cell type). A significant positive correlation between NSE levels and the survival periods was found in follow-up studies of 10 patients who underwent surgery with complete resection of the primary tumor. All of 4 long term survivors over 3 years after surgery had significantly high NSE and relatively low CEA producing tumors. The relationship of these markers to clinical status of the patient suggests that an analysis for correlation of NSE and CEA levels in SCLC patients may be useful to discriminate between a pure neuroendocrine SCLC tumor and a mixed small cell/large cell tumor, and in monitoring therapeutic effect and prognosis of each patient. —— NSE; CEA; SCLC; tumor heterogeneity; cell line

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Although, small-cell lung cancer (SCLC) is highly responsive to chemotherapy and radiotherapy, it is an aggressive disease and has a poor prognosis with 3 year disease-free survival being only 5–10% (Choi et al. 1987; Souhami and Law 1990). The main reason for this outcome is the presence of non-small cell elements resistant to chemotherapy within the primary tumor of SCLC (Matthews 1979). An autopsy study on patients diagnosed as SCLC by biopsy showed exclusively non-SCLC in 5.5–12.5% and combined mixed tumors in 14.3–32.9% after intensive chemotherapy (Abeloff and Eggleston 1981). Furthermore, many authors have described that patients with mixed small cell/large cell tumors have a considerably worse prognosis and lower treatment response rate than those with pure SCLC (Radice et al. 1982; Weynants et al. 1990). In these clinical manifestations, the basic study on morphologic and biologic heterogeneity of SCLC in vitro has been considered as the considerable important subject in recent years. Tumor markers in culture media of cancer cell lines express clearly the differentiation for biological characteristics of each cell line in vitro, and are considered to be useful to analyze the heterogeneity of SCLC in vitro.

As described in previous reports, we had devised a technique of establishing tumor cell lines and established a number of pulmonary carcinoma cell lines (Kobayashi et al. 1989; Kobayashi and Fujimura 1992). Although the examination of a large comprehensive panel of cell lines are necessary to study the correlation between biological characteristics and clinical manifestation of lung cancer, in the literature, the number of basic studies that examined tumor marker production in vitro using various human pulmonary carcinoma cell lines is very small. Therefore, within the framework of basic studies using a number of the cell lines which we have established, we had examined the levels of several tumor markers in culture media of human pulmonary carcinoma cell lines (Kobayashi et al. 1994) and found that neuron-specific enolase (NSE) and carcinoembryonic antigen (CEA) are useful markers to analyze the heterogeneity of SCLC cells.

Materials and Methods

Cell lines and cultures

As shown in Table 1, this study used the following 65 human pulmonary carcinoma cell lines which were consecutively established in our laboratory from tumor specimens obtained from patients admitted to our hospital between 1976 and 1989: 18 patients with small cell carcinoma, 14 patients with large cell carcinoma, 14 with squamous cell carcinoma, 14 with adenocarcinoma and 5 with adenosquamous cell carcinoma. Diagnosis of these patients were confirmed on tissue sections obtained at thoracotomy, bronchoscopy and lymph node biopsy by staining with hematoxylin and eosin. Histopathological classification was carried out in accordance with the revised World Health Organization (WHO) classification of lung tumors (WHO 1982). All of the cell lines had been maintained in Ham F12 medium supplemented with 5% fetal bovine serum for at

least 3 years and stored by liquid N_2 before being tested. The cells used for the primary culture of pulmonary carcinoma cells were collected from surgically removed primary tumors in 46 patients, from malignant pleural effusion in 1,

TABLE 1. *Profiles of 65 human pulmonary carcinoma cell lines*

Cell line	Age, Sex	Primary culture	Vitro D.T. (Days)	Hist.	Cell line	Age, Sex	Primary culture	Vitro D.T. (Days)	Hist.
76-1	62, M	Tumor	6	Sq	87-3	80, M	MLN	14	Ad
76-2	63, M	Tumor	0.9	Ad	87-4	76, M	Tumor	9.2	Sm
76-3	63, M	Tumor	1.7	Ad	87-5	64, M	MLN	1.8	Sm
77-1	59, M	MLN	1.4	Ad	87-6	67, M	Tumor	6.7	Ad
77-2	53, F	Pl. Effus.	28	Ad	87-9	66, M	Tumor	8	Sq
77-3	68, M	Tumor	6	AdSq	87-10	58, M	MLN	4.8	L
77-4	67, F	Tumor	1.9	Ad	87-11	70, M	Tumor	6.5	L
78-2	44, M	MLN	1.8	Sm	87-12	57, M	MLN	2	L
81-1	49, F	Tumor	4.4	Ad	87-13	67, F	Tumor	11.7	Ad
83-1	67, M	Tumor	2.2	Sm	87-14	65, F	Tumor	21	Sm
83-2	53, M	SCM	10	Sm	87-17	57, M	SCM	3.7	L
83-3	67, M	MLN	6	Sm	87-20	51, M	Tumor	12.4	L
84-1	69, M	MLN	0.8	Sm	88-1	65, M	Tumor	4.2	Ad(Sq)
84-2	61, M	Tumor	1.3	Sm	88-2	47, M	Tumor	1.5	Ad
84-3	63, M	Tumor	9	Sq	88-3	63, F	Tumor	1.7	Ad
84-5	54, M	MLN	0.9	Sm	88-4	60, F	Tumor	1.7	Ad
85-1	70, M	Tumor	0.9	Sq	88-5	62, M	Tumor	2	Sq
85-2	59, M	Tumor	4	Sq	88-6	58, M	Tumor	6	L
85-3	68, M	Tumor	2.9	Ad	88-7	64, M	Tumor	7	Sm
85-4	59, M	Tumor	6	Sq	88-8	74, M	Tumor	3.2	Sm
85-5	61, M	Tumor	2.1	L	88-9	63, M	MLN	2.4	Sm
86-1	66, M	Tumor	7.1	Sq	88-10	51, M	Tumor	7	L
86-2	36, M	Tumor	1.5	L	88-12	64, M	MLN	13.5	L
86-5	58, M	Tumor	10	Sq	88-13	38, M	Tumor	2	L
86-6	65, M	Tumor	17.7	AdSq	88-14	63, M	Tumor	4.1	Sq
86-7	51, M	Tumor	6.7	AdSq	88-17	73, M	MLN	5.7	Sm
86-8	67, M	Tumor	5.8	Sq	88-19	66, M	MLN	8.7	L
86-9	68, M	MLN	5.9	Sm	88-24	67, M	Tumor	6.8	L
86-10	56, M	SCM	7	Sq	88-27	72, M	Tumor	9.8	Sm
86-11	62, M	Tumor	1	Sq	88-29	69, M	Tumor	4.7	L
86-14	65, M	Tumor	5	AdSq	89-1	63, M	Tumor	14	Sm
87-1	52, M	Tumor	12.1	Ad	89-3	56, M	MLN	7.2	Sm
87-2	67, M	Tumor	5.4	Sq					

MLN, metastatic lymph node; SCM, subcutaneous metastasis; Pl. Effus., pleural effusion; D.T., doubling time; Hist., histology; L, large cell carcinoma; AdSq, adenosquamous cell carcinoma; Ad(Sq), adeno and squamous double cancer; Ad, adenocarcinoma; Sq, squamous cell carcinoma; Sm, small cell carcinoma.

subcutaneous metastatic tumor in 3 and from mediastinal or cervical lymph node metastases in 15 patients (Table 1). Cells were cultured by a modification of the method for short-term selective cultivation we had previously developed. The details of this technique have been reported elsewhere (Kobayashi et al. 1989).

Determination of NSE and CEA levels in culture media

Each cell line was suspended in culture medium (2.5×10^5 cells/ml) with 0.25% trypsin solution. Two ml of each cell suspension (5×10^5 cells) was transferred to each well of a 24-well multiplate. Half the total volume of each culture medium was replaced with fresh medium weekly. Four days after medium renewal which was carried out after 4 weeks from passage, we collected 1 ml of the culture supernatants to measure NSE and CEA levels, since NSE and CEA levels in culture media after 4 weeks period of cultivation amount to almost plateau on time-dose curve line in some pulmonary carcinoma cell lines (preliminary data). NSE and CEA quantification were done using commercially available radio-immunoassay kits (Dainabot, Tokyo; Pharmacia, Uppsala, Sweden).

Survival analysis

Of 18 SCLC patients, 10 with stage I–IIIa diseases who underwent surgery with complete resection of primary tumor were examined their prognostic outcome. Staging was based on the international staging system (Mountain 1986). For all these patients, survival was measured from the date of operation until the date of death or the last follow-up date of 60 months after surgery. Survival curves were calculated by a standard life table methods.

Statistical analysis

The data were statistically evaluated by non-parametric procedures using the Man-Whitney U-test, taking $p<0.05$ as the level of significance.

Results

NSE levels in supernatant of SCLC and non-SCLC

As shown in Table 2, the NSE level in the supernatant of the cultures of pulmonary carcinoma cell lines varied from 2 to 84 ng/ml. The mean value of NSE in media was 30.8 ± 22.4 ng/ml (range, 6–84 ng/ml; median 24.5 ng/ml) and 9.2 ± 8.7 ng/ml (range, 2–49 ng/ml; median 6.0 ng/ml) in SCLC and non-SCLC, respectively. A significant difference was obtained between the mean values of NSE in the SCLC lines and non-SCLC lines when they were compared ($p<0.01$). When the cutoff level of NSE was set at 10 ng/ml (a level usually used as a cutoff level of human serum NSE), 15 of the 18 SCLC lines (83.3%) and 17 of 47 non-SCLC lines (36.2%) had higher than the cutoff level. The distribution of the NSE levels in relation to the histological types of non-SCLC is shown in Fig. 1. Ten of 14 large cell carcinoma cell lines (71.4%), 6 of 14 squamous cell carcinoma

TABLE 2. *NSE and CEA levels in the culture media of 65 human pulmonary carcinoma cell lines*

	No.	NSE (ng/ml)		CEA (ng/ml)	
		Mean±S.D. (Range)	Median	Mean±S.D. (Range)	Median
Small cell carcinoma	18	30.8±22.4 (6-84)	24.5	15.1±20.9 (0.5- 65)	2.8
Non-small cell carcinoma	47	9.2± 8.7 (2-49)	6.0	26.6±72.3 (0.5-410)	1.5
Large cell	14	14.5±12.3 (2-49)	12.0	23.7±74.1 (0.5-290)	0.7
Squamous	14	9.7± 6.4 (2-24)	7.6	14.9±24.8 (0.5- 86)	1.5
Adeno & Adsq	19	4.9± 2.4 (2-12)	4.4	37.4±90.6 (0.5-410)	7.3

NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; Adsq, adenosquamous cell carcinoma.

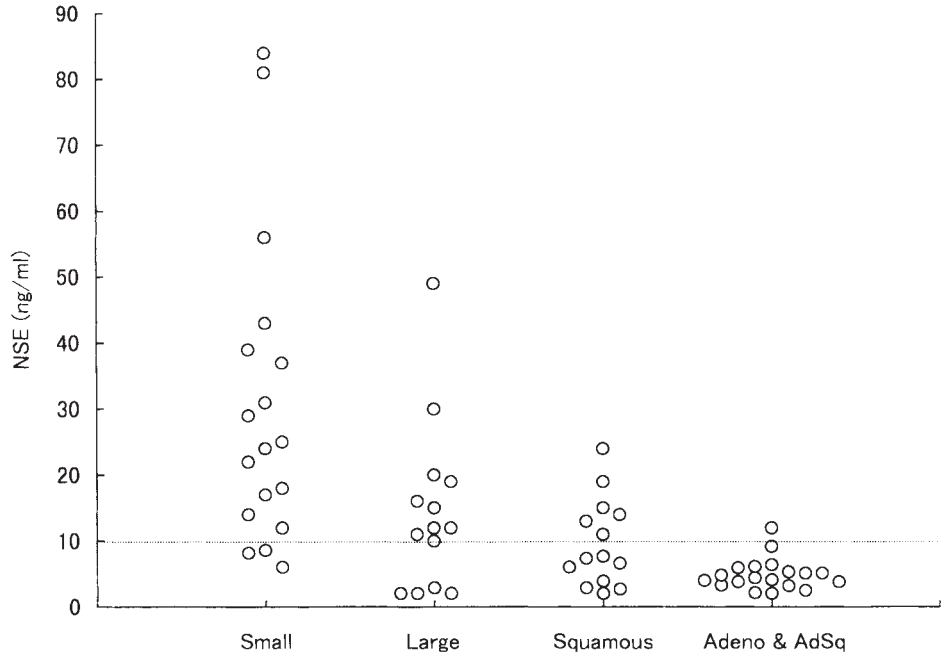

Fig. 1. NSE levels in each histological type of pulmonary carcinoma.
Ten of 14 large cell carcinoma cell lines (71.4%), 6 of 14 squamous cell carcinoma cell lines (42.9%), and 1 of 5 adenosquamous cell lines (20%) had medium NSE levels higher than the cutoff level of 10 ng/ml, whereas none of 14 adenocarcinoma cell lines had elevated NSE levels.

cell lines (42.9%), and 1 of 5 adenosquamous cell lines (20%) had medium NSE levels higher than the cutoff level of 10 ng/ml, whereas none of 14 adenocarcinoma cell lines had elevated NSE levels. The mean value for large cell carcinomas, squamous cell carcinomas, and adenocarcinoma and adenosquamous cell carcinomas were 14.5±12.3, 9.7±6.4 and 4.9±2.4 ng/ml, respectively. The NSE level in large cell carcinoma was significantly different from that in squamous cell carcinoma ($p<0.05$), and adenocarcinoma and adenosquamous cell carcinoma ($p<0.01$).

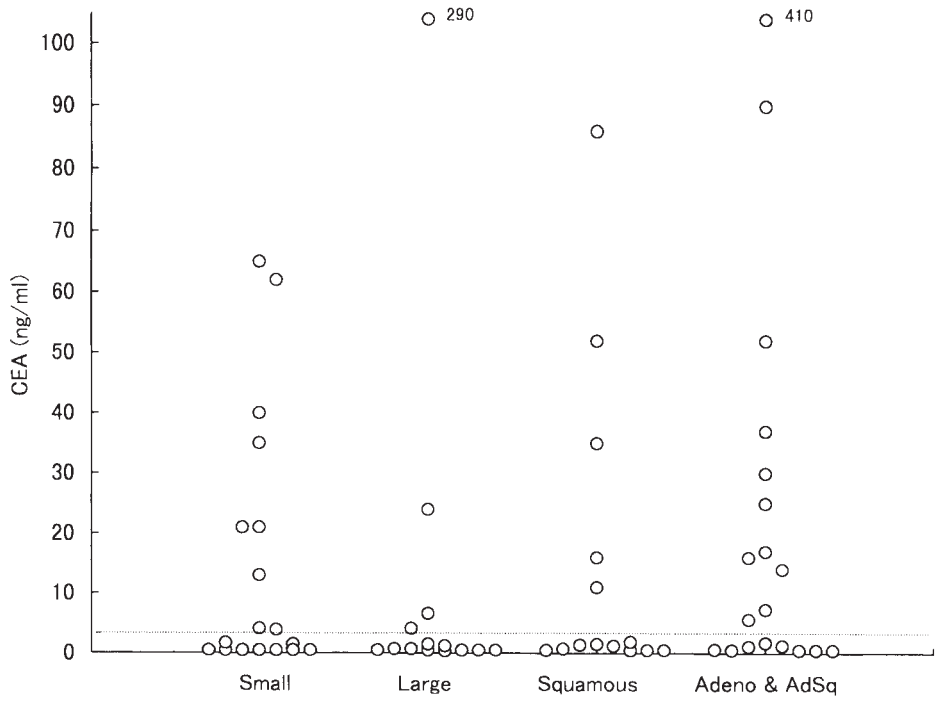

Fig. 2. CEA levels in each histological type of pulmonary carcinoma.
When the cutoff level was set at 2.5 ng/ml, the CEA-positive rate was 71% (10/14) for adenocarcinoma, 50% (9/18) for small cell carcinoma, 36% (5/14) for squamous cell carcinoma, 29% (4/14) for large cell carcinoma and 20% (1/5) for adenosquamous cell carcinoma.

CEA levels in supernatant of SCLC and non-SCLC

CEA levels in the supernatant of pulmonary carcinoma cell lines also varied that ranged from 0.5 to 410 ng/ml as shown in Fig. 2. The mean value of CEA in SCLC and non-SCLC in the media was 15.1 ± 20.9 ng/ml (range, 0.5–65 ng/ml; median 2.8 ng/ml) and 26.6 ± 72.3 ng/ml (range, 0.5–410 ng/ml; median 1.5 ng/ml), respectively. The mean value of non-SCLC was higher than SCLC because of extremely high levels in several cell lines of non-SCLC. However, the median value did not show a definite difference among SCLC lines and non-SCLC lines. The CEA levels in the supernatant depending on the histological type were shown in Table 2 and Fig. 2. When the cutoff level was set at 2.5 ng/ml, the CEA-positive rate was 71% (10/14) for adenocarcinoma, 50% (9/18) for small cell carcinoma, 36% (5/14) for squamous cell carcinoma, 29% (4/14) for large cell carcinoma and 20% (1/5) for adenosquamous cell carcinoma (Fig. 2). There were no significant differences between CEA levels in SCLC and non-SCLC cell lines. Massive CEA production (over 100 fold of cutoff level) was noted in one cell line each of large cell carcinoma and adenocarcinoma, but not small cell carcinomas.

Correlation between NSE and CEA levels of individual 18 SCLC cell lines

When NSE and CEA levels in each of 18 SCLC lines were analyzed, there was a significant inverse proportional correlation between NSE and CEA production

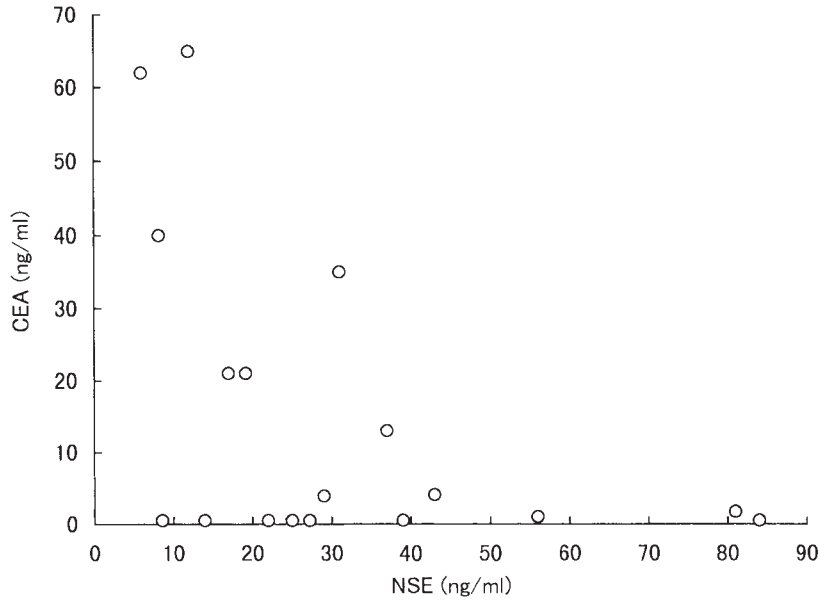

Fig. 3. Correlation between NSE and CEA levels of individual 18 SCLC cell lines.
When NSE and CEA levels in each of 18 SCLC lines were analyzed, there was a significant inverse proportional correlation between NSE and CEA production ($r=-0.473$, $p<0.05$).

($r=-0.473$, $p<0.05$) (Fig. 3). The CEA production tended to be higher in cells with low levels of NSE than in those with high NSE production.

Therefore, we next evaluated the relationship between the growth characteristics in vitro and the quantitative level of each of the two markers. In this analysis, when CEA levels in culture media increased, while NSE levels decreased, the cells had a more tendency toward attachment to the basal surface. Fig. 4 shows phase microscope pictures of 6 small cell carcinoma cell lines, which produced various amounts of NSE and CEA in the supernatant. With respect to correlation between NSE and CEA content, high NSE and low CEA production was found in cells growing with floating colony type or neurite like cells (classic cell type); low NSE and high CEA in cells with epithelial type or in the cells showing intermediate growth characteristics (variant cell type). As shown in Table 3, the mean value of NSE levels in 11 cell lines with classic type and 7 with variant cell type was 39.9 ± 23.8 ng/ml (range, 8.6–84 ng/ml) and 16.6 ± 8.18 ng/ml (range, 6–31 ng/ml), respectively ($p<0.05$). The mean value of CEA in cells with classic type and with variant cell type was 2.43 ± 3.59 ng/ml (range, 0.5–13 ng/ml) and 34.9 ± 21.5 ng/ml (range, 0.5–65 ng/ml), respectively ($p<0.01$).

Correlation between NSE and CEA levels and the survival of the patients

To determine the possible clinical significance of tumor markers in SCLC, relationship between NSE and CEA levels in supernatant of SCLC lines was analyzed according to the survival period of each case. Eight of 18 SCLC lines tested were advanced cases with cStage IIIb or IV. Therefore, we analyzed 10

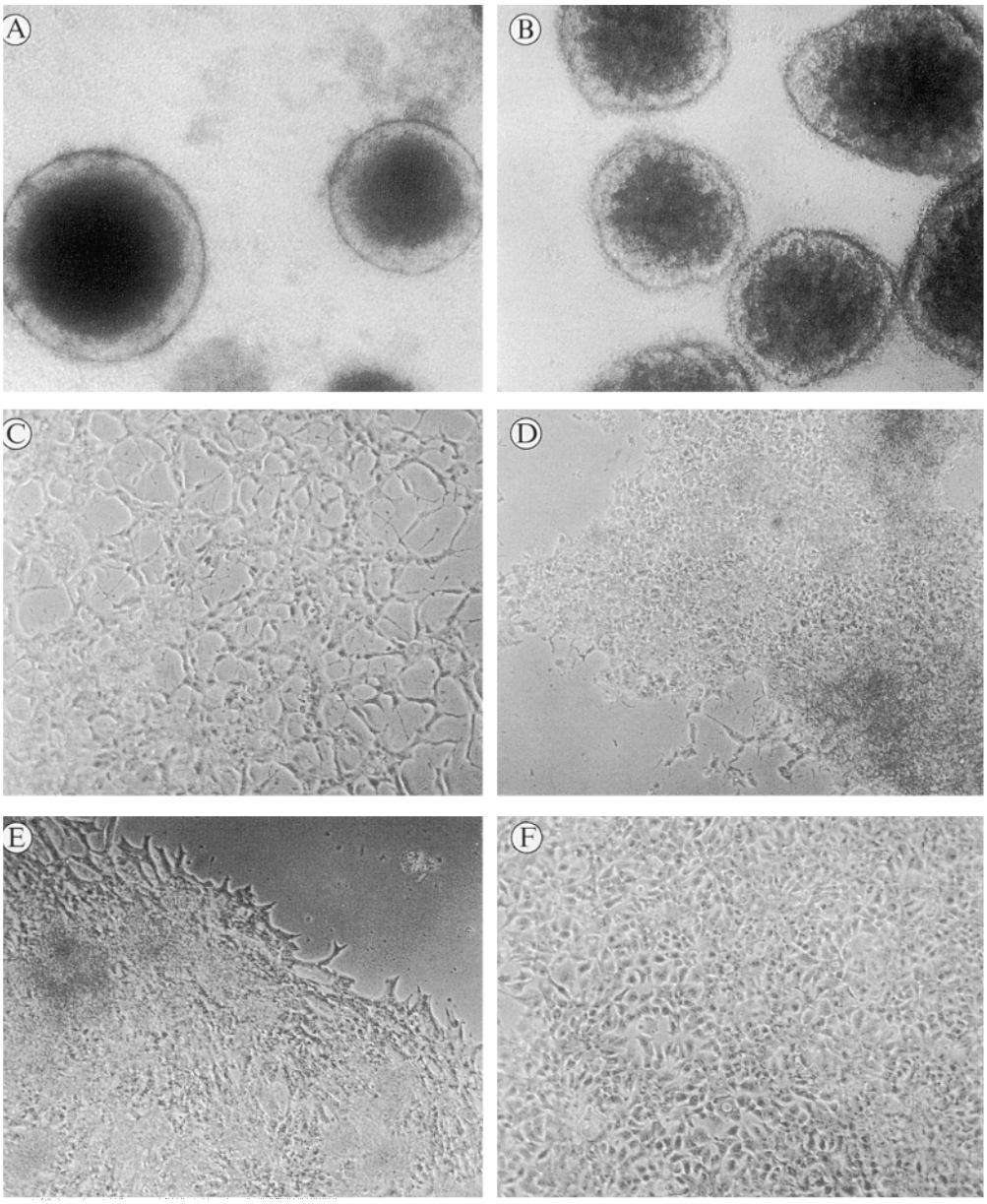

Fig. 4 The relationship between the growth characteristics in vitro and the quantitative level of the NSE and CEA markers in each SCLC cell line.
A. 83-3: NSE 39, CEA 0.5, B. 87-4: NSE 56, CEA 1.0, C. 84-1: NSE 22, CEA 0.5, D. 83-1: NSE 31, CEA 34, E. 86-9: NSE 12, CEA 66, F. 88-9: NSE 6, CEA 62.
High NSE and low CEA production was found in cells growing with floating colony type (A, B) or neurite like cells (C); low NSE and high CEA in cells with epithelial type (F) and intermediate production of each marker in the cells showing intermediate growth characteristics (D, E). (Phase contrast micrograph $\times 100$)

cases with pStage I–IIIa who received surgical treatment with complete resection of the primary tumor. The survival period of these patients after surgery ranged from 11 to over 60 months (3 year disease free survival: 4/10, 40%). As shown in Table 3, the effect of an increased proportion of CEA levels is aggressive, while that of NSE is not. There was a significant positive correlation between the NSE

TABLE 3. *NSE and CEA levels in the culture media of 18 SCLC cell lines*

Cell line	Age, Sex	Date of culture	Operation	pTNM	Path. Stage	Path. Type	Prog. (month)	Growth Pattern	NSE (ng/ml)	CEA (ng/ml)
87-4	76 M	87/02/26	RUL	T1N0M0	I	Int.	>60	Floating	56	1
88-27	72 M	88/12/08	LLL + TW	T3N0M0	IIIa	Int.	>60	Floating	84	0.5
89-1	63 M	89/02/02	LLL	T1N0M0	I	Int.	>60	Neurite	29	3.9
83-2	53 M	83/07/04	RUL	T1N0M0	I	Int.	51.4	Floating	81	1.7
83-1	67 M	83/02/01	LP	T1N0M0	I	Oat	24.2	Intermed.	31	35
86-9	68 M	86/10/23	RLL	T1N0M0	I	Int.	23	Intermed.	12	65
88-7	64 M	88/03/10	RLL	T2N1M0	II	Int.	16.7	Floating	25	0.5
87-14	65 F	87/10/13	LP+LA	T3N2M0	IIIa	Oat	14.9	Intermed.	18	21
88-8	74 M	88/03/29	(ICT)LUL	T2N1M0	II	Oat	11	Intermed.	17	21
84-2	61 M	84/09/11	RUL	T2N0M0	I	Int.	10	Epithelial	8.2	40
83-3	67 M	83/11/09	(−)	M1	IV	Oat		Floating	39	0.5
87-5	64 M	87/03/04	(−)	N3M0	IIIb	Int.		Floating	37	13
88-17	73 M	88/10/11	(−)	M1	IV	Int.		Floating	43	4.1
78-2	44 M	78/08/22	(−)	M1	IV	Int.		Neurite	8.6	0.5
84-1	69 M	84/01/19	(−)	M1	IV	Oat		Neurite	22	0.5
89-3	56 M	89/07/18	(−)	N3M0	IIIb	Int.		Neurite	14	0.5
84-5	54 M	84/11/24	(−)	N3M0	IIIb	Int.		Intermed.	24	0.5
88-9	63 M	88/04/20	(−)	M1	IV	Oat		Epithelial	6	62

NSE, neuron-specific enolase; CEA, carcinoembryonic antigen; Path., pathological; RUL, right upper lobectomy; RLL, right lower lobectomy; LUL, left upper lobectomy; LLL, left lower lobectomy; LP, left pneumonectomy; TW, thoracic wall; LA, left atrium; ICT, induction chemotherapy; Int., intermediate cell type; Oat, oat cell type; Prog., prognosis (survival from the date of operation to death); pTNM, pathologic TNM classification (Mountain 1986); Growth pattern, see text.

levels and survival ($r=0.789$; $p<0.01$). All of 4 long term survivors over 3 years after surgery had high NSE (mean: 62.5, range: 29–84) and low CEA (mean: 1.8, range: 0.5–3.9) producing tumors. On the other hand, six patients died within 3 years had low NSE (mean: 18.5, range: 8–31) and high CEA (mean: 30.4, range: 0.5–65) producing tumors. A statistically significant high NSE level was found in the culture media of cells from 4 long term survivors ($p<0.05$). The postoperative median survival time was 15 months for 5 patients with high CEA producing tumors (greater than 10 ng/ml) and over 60 months for the 5 patients with lower CEA levels ($p<0.05$).

DISCUSSION

Small-cell lung cancer (SCLC) is a common malignancy that is usually fatal in spite of a combined modality therapy with surgical treatment, since it early metastasizes and recurs even after aggressive chemotherapy because of drug

resistant cells regrowing. The cellular origin of this cancer is not well established, though the cells of many SCLC tumors express a variety of neuroendocrine markers (Moody et al. 1981; Marangos et al. 1982). Several theories suggest that lung carcinomas are derived from a common precursor of endodermal origin, because there is considerable overlap of biological properties between non-SCLC and SCLC (Gazdar et al. 1981; Yesner 1985). In addition, the cellular and histological heterogeneity with non-SCLC elements among SCLC has been the subject of considerable interest during the last decade.

In our present study, NSE production was prominent and significantly elevated in SCLC cell lines, but was not limited to them. The present data examined in a number of cell lines have clearly confirmed that NSE was commonly produced in non-SCLC cell lines, especially large cell carcinoma. These results are coincident with serum tumor marker analysis (Ariyoshi et al. 1983) and in vitro data by others whose studies suggest that non-SCLC cells express neuroendocrine properties in 10% to 20% of cases (Gazdar et al. 1988). Furthermore, Linnoila et al. (1994) described that 46% tumors was positive for NSE marker by immunohistochemistry in 237 resected non-SCLCs, and he suggested that these neuroendocrine features in non-SCLC may demonstrate biological behavior intermediate between non-SCLC and SCLC with increased responsiveness to chemotherapy. In contrast to NSE content, the level of CEA was not prominent in SCLC cells in vitro. However, the present study also showed that higher levels of the production of CEA are common among SCLC lines, when compared with that of normal serum cutoff level of 2.5 ng/ml. These results are also coincident with serum tumor marker analysis by others (Jaques et al. 1988; Plebani et al. 1995)

In our present study, it was clearly demonstrated that such NSE and CEA levels revealed an inverse correlation in individual SCLC lines, and had an important influence on its growth characteristics of cells in vitro. High NSE levels were found predominantly in cell lines with floating colony formation or neurite cells and high CEA levels in epithelial cells as shown in Fig. 4. The data presented here also suggest a measurement of NSE and CEA levels may be useful for monitoring non-SCLC differentiation in SCLC tumor and the clinical course of the patient, because most non-SCLC cells grow attached to the substrate and most SCLC cells grow as floating aggregates in early subcultures as described in our previous reports (Kobayashi et al. 1989; Kobayashi and Fujimura 1992) and others (Carney et al. 1985). Furthermore, Gazdar et al. (1985) report that SCLC cell lines can be classified into two major groups of classic cell lines with floating cell aggregates and variant cell lines which morphologically resemble large cell carcinoma and grow attached to the substrate. Therefore, it was considered that the attached cells with epithelial or intermediate growth characteristics in our study were the same as non-SCLC like variant cells prescribed by others (Carney et al. 1983; Gazdar et al. 1985; Gazder and Oie 1986). Accordingly, the correla-

tion between NSE and CEA levels have better predictive value for the amount of non-SCLC-like variant cells among the SCLC tumor.

Our these present data were contrary to the results published by Bepler et al. (1987, 1989), who found CEA in 60% of classic SCLC cell lines but none of the variant SCLC cell lines and non-SCLC cell lines. In our study, higher CEA production is, however, clearly observed in SCLC cell lines exhibiting variant type and also a number of non-SCLC cell lines than in classic SCLC cells with floating colony or neurite cell formation. The reason of this discrepancy is most likely a result of the difference of measuring materials and methods. Bepler et al. (1989) measured the CEA levels in cell homogenates of cultured cells using enzyme immunoassay (EIA). On the other hand, we have measured the CEA levels in supernatant of cultured cells by using RIA technique, because we considered that the levels of spontaneous soluble CEA release from cells into culture media most likely reflect the CEA levels in serum of patients. Recently, it is known that serum CEA levels result from several factors, including not only cellular CEA content but also the rate of CEA release (Gouin et al. 1993), and therefore we suppose that total cellular CEA contents measured by Bepler et al. (1989) are not parallel to serum CEA levels in clinical course of the patients.

In the present study, while numbers of tested SCLC cell lines in relation to their survival are small, it is clear that the majority of cases with cell line with production of low CEA and high NSE levels survived over 3 years after surgical treatment. In contrast, the cases with cell lines which produce low NSE and high CEA levels in the supernatant were poor prognosis. A possible explanation for the poor prognosis in patients with high CEA and low NSE produced cell lines could be that; high levels of production for CEA and low levels for NSE in SCLC denotes that CEA producing non-SCLC like variant cells were included among initial SCLC tumor, and therefore the patient with these heterogenous SCLC tumor had a worse prognosis because the CEA producing non-SCLC like variant cancer cells was generally resistant to anticancer drugs and radiation (Carney et al. 1983; Kobayashi et al. 1989). These epithelial cell lines occasionally change to large cell carcinoma in histological appearance of nude mouse transplanted tumor (Kobayshi et al. 1989), which also resemble that drug resistant non-SCLC cells among SCLC tumors are left after intensive chemotherapy and occasionally regrow as a tumor with histological appearance of a large cell carcinoma in clinical experience. Waalkes et al. (1980) reported that a rising CEA level in patients' sera with SCLC was usually found with recurrence or progression of disease after initial response of chemotherapy and occurred frequently prior to clinical evidence of progression. It is supposed that this morphological shift to large cell carcinoma is accompanied by a decreased expression of NSE and a increased expression of CEA.

In conclusion, it could thus be demonstrated that the SCLC cell lines with different growth characteristics in vitro differ in their ability to express NSE and

CEA. This may reflect the different in origin of the respective tumor groups, cellular heterogeneity of the tumors and the clinical behaviors. Presence of CEA producing cells among SCLC tumor and a rising CEA level in serum may be able to be used as a prognostic factor in patients with SCLC, though it will require further study on many patients using multifactorial analysis. The relationship of these markers to survival of the patient suggests that an analysis for correlation of NSE and CEA levels in SCLC patients may be useful to discriminate between a pure neuroendocrine SCLC tumor and a mixed small cell/large cell tumor with heterogeneity, and in monitoring therapeutic effect and prognosis of each patient.

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