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Clinical responses of large cell neuroendocrine carcinoma of the lung to cisplatin-based chemotherapy

Shigeo Yamazaki[a,d], Ikuo Sekine[a,*], Yoshihiro Matsuno[b], Hidefumi Takei[c], Noboru Yamamoto[a], Hideo Kunitoh[a], Yuichiro Ohe[a], Tomohide Tamura[a], Tetsuro Kodama[a], Hisao Asamura[c], Ryosuke Tsuchiya[c], Nagahiro Saijo[a]

[a] *Division of Thoracic Oncology and Internal Medicine, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan*
[b] *Division of Clinical Laboratory, National Cancer Center Hospital, Tokyo*
[c] *Division of Thoracic Surgery, National Cancer Center Hospital, Tokyo*
[d] *Department of Surgery, Keiyu-kai Sapporo Hospital, Sapporo, Japan*

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KEYWORDS
Neuroendocrine carcinoma;
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Summary
Background: The efficacy of chemotherapy in patients with large cell neuroendocrine carcinoma of the lung (LCNEC) remains unclear.
Methods: Patients with LCNEC who received cisplatin-based chemotherapy were identified by reviewing 567 autopsied and 2790 surgically resected lung cancer patients. The clinical characteristics and objective responses to chemotherapy in these patients were analyzed.
Results: Overall, 20 cases of LCNEC were identified, including stage IIIA ($n=3$), stage IIIB ($n=6$), stage IV ($n=6$) and postoperative recurrence ($n=5$) cases. Six patients had received prior chemotherapy, and 14 were chemo-naive patients. The patients had received a combination of cisplatin and etoposide ($n=9$), cisplatin, vindesine and mitomycin ($n=6$), cisplatin and vindesine ($n=4$), or cisplatin alone ($n=1$). One patient showed complete response and nine showed partial response, yielding an objective response rate of 50%. The response rate did not differ between patients with the initial diagnosis of SCLC and those with the initial diagnosis of NSCLC, however, the response rate in chemo-naive patients (64%) was significantly different from that in previously treated patients (17%).
Conclusions: Our results suggest that the response rate of LCNEC to cisplatin-based chemotherapy was comparable to that of SCLC.

* Corresponding author. Tel.: +81 3 3542 2511; fax: +81 3 3542 3815.
E-mail address: isekine@ncc.go.jp (I. Sekine).

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1. Introduction

Pulmonary neuroendocrine tumors include a spectrum of four clinicopathological entities classified on the basis of the morphological and biological features: typical carcionoid and atypical carcinoid, which are tumors of low to intermediate grade malignancy, and large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC), which are high-grade malignant tumors. Travis et al. proposed the term LCNEC in 1991 [1], for classifying a type of poorly differentiated high-grade carcinoma characterized by a neuroendocrine appearance under light microscopy. LCNEC exhibits more prominent cellular pleomorphism and higher mitotic activity than the atypical carcinoid (AC), and is distinguished from SCLC by the tumor cell size and chromatin morphology. Although several different terminologies and classifications have been proposed previously, and even the present classification of pulmonary neuroendocrine tumors lacks uniform definition criteria, this class of tumors could become widely accepted and included in the updated histological classification of the World Health Organization [2].

The clinical features of LCNEC have not yet been completely clarified. The prognosis of patients with surgically resected LCNEC is reported to be intermediate between that of AC and SCLC [3–5], and the same as that of resected NSCLC, except that stage I LCNEC has a poorer prognosis than stage I non-small cell lung cancer (NSCLC) [6]. To the best of our knowledge, however, there are no studies that have examined the role of chemotherapy for LCNEC and the prognosis of patients with unresectable LCNEC, even though several reports have been published on the association between response to chemotherapy and the neuroendocrine differentiation of NSCLC [7–9]. The appropriate treatment of unresectable LCNEC, therefore, remains unclear. In the present study, we attempted to investigate the effectiveness of chemotherapy with cisplatin-based regimens for LCNEC in patients with unresectable and recurrent LCNEC.

2. Materials and methods

Eighty-seven of 2790 patients with primary lung cancer who underwent tumor resection from 1982 to 1999 at the National Cancer Center Hospital were found to have tumors with the histological characteristics of LCNEC [6]. Of these, five had received cisplatin-based chemotherapy at the time of recurrence, and were enrolled as subjects of this study. In addition, 303 of 567 patients who were autopsied from 1983 to 1997 at the National Cancer Center Hospital who had the following histological diagnoses were first selected: SCLC ($n=112$), poorly differentiated adenocarcinoma ($n=99$), large cell carcinoma ($n=58$), poorly differentiated squamous cell carcinoma ($n=29$), poorly differentiated adenosquamous carcinoma ($n=2$), LCNEC ($n=2$), and carcinoid ($n=1$). Of these, 161 had received cisplatin-based chemotherapy were selected for a pathological review. Finally, specimens from 17 of these161 cases were found to have histological characteristics consistent with the diagnosis of LCNEC, and were selected as subjects of this study. We focused on cisplatin, because since the 1980s, cisplatin has been the only anticancer agent with proven efficacy against both SCLC and NSCLC [10,11]; we, therefore, considered that the effectiveness of chemotherapy for LCNEC could be reasonably evaluated if cisplatin were included in the regimen. Cases which had received adjuvant chemotherapy without evaluable lesions were excluded from the analysis.

All the available paraffin-embedded tissue sections stained with hematoxylin–eosin were reviewed. We classified LCNEC according to the histopathological criteria in the WHO classification [2]. Immunohistochemical analysis was performed to confirm the neuroendocrine features of the tumors. For this purpose, formalin-fixed paraffin sections were stained for a panel of neuroendocrine markers, including chromogranin A (CGA), synaptophysin (SYN), and neural cell adhesion molecule (NCAM), using standard methods. The intensity of immunostaining for these markers was scored as follows: +, when the proportion of stained tumor cells was >50%; ±, when 10–50% of tumor cells were stained; and −, when <10% of tumor cells were stained, as previously described [6]. One case included in this study had the typical histological features of LCNEC, but no neuroendocrine features as determined by the immunohistochemical analysis. For specimens obtained after treatment, we routinely confirmed that the histopathological and morphological features showed no changes due to treatment as compared with the pretreatment biopsy or cytologic specimens. Such cases for which no pretreatment samples were available were excluded from the study; since it has been reported that histological changes may occur after treatment in SCLC [12], we were concerned that misdiagnosis might occur if the same were also true for LCNEC.

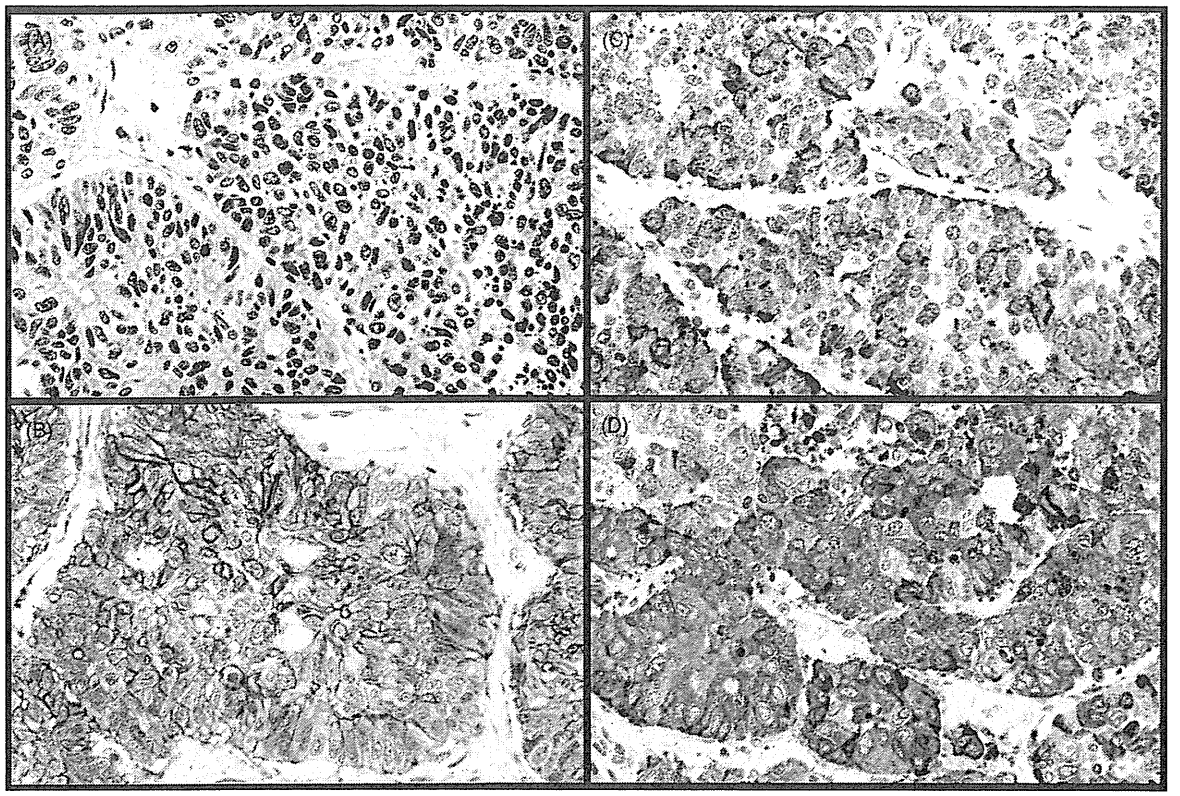

Fig. 1 Case no. 2, 57-year-old man. (A) The tumor cells which are large-sized, polygonal in shape and have a low nuclear-cytoplasmic ratio, are arranged in organoid nests and trabeculae (H&E stain, ×200). Positive staining for neural cell adhesion molecule (B), chromogranin A (C), and synaptophysin (D) (immunostain, ×400).

Clinical information about the cases was obtained from the medical records. The clinical disease staging was reassessed according to the latest International Union Against Cancer (UICC) staging criteria [13]. The response to chemotherapy and overall survival rate were assessed retrospectively. The objective tumor response was evaluated according to the WHO criteria published in 1979 (WHO, 1979) [14]. The survival time was measured from the date of start of chemotherapy with a cisplatin-containing regimen. Survival curves were drawn using the Kaplan–Meier method [15]. Drug toxicity could not be assessed as the study was a retrospective one and records were often incomplete.

3. Results

Overall, 22 cases were recognized as having tumors with histological characteristics consistent with LCNEC among the autopsied and surgically resected cases of primary lung cancer that had received cisplatin-based chemotherapy and had evaluable lesions; of these 17 were autopsied cases and five were surgically resected cases. Two of the autopsied cases were excluded, because no pretreatment pathological or cytological samples were available. The typical microscopic appearance of the tumor specimens is shown in Fig. 1A. The specimen sources for the prechemotherapy-diagnosis included surgically resected specimens ($n = 5$), biopsy specimens ($n = 9$), and cytology specimens ($n = 6$). The histological and cytological findings in the specimens obtained before chemotherapy were consistent with those in the specimens obtained after chemotherapy. We therefore finally enrolled 20 cases in this study. The initial pathologic diagnoses in these patients were as follows: small cell carcinoma ($n = 10$), poorly differentiated adenocarcinoma ($n = 6$), large cell carcinoma ($n = 2$), undifferentiated carcinoma ($n = 1$), and poorly differentiated carcinoma ($n = 1$) (Table 1). None of the cases had been labeled as LCNEC at the time of initial diagnosis, probably because the concept of LCNEC

Table 1 Patient characteristics

Characteristics	N	%
No. of patients	20	
Sex		
Male	18	90
Female	2	10
Age, median (range)	58 (37–74)	
Smoking history		
Yes	19	95
No	1	5
Performance status		
1–2	19	95
>2	1	5
Initial pathological diagnosis		
Small cell carcinoma	10	50
Adenocarcinoma	6	30
Large cell carcinoma	2	10
Others	2	10
Clinical stage at the start of chemotherapy		
IIIA	3	15
IIIB	6	30
IV	6	30
Postoperative recurrence	5	25
Prior treatment		
None	10	50
Surgery	4	20
Radiotherapy	2	10
Chemotherapy without cisplatin	6	30

Table 2 Staining for neuroendocrine markers in 20 LCNECs

Case	NCAM	CGA	SYN
1	+	+	+
2	+	+	+
3	+	+	+
4	±	+	+
5	+	+	+
6	+	+	+
7	−	+	−
8	+	−	−
9	−	−	−
10	−	+	±
11	+	−	+
12	+	+	+
13	+	+	+
14	+	−	±
15	+	+	+
16	+	−	NA
17	+	−	+
18	+	−	NA
19	+	−	+
20	−	+	+

NCAM, neural cell adhesion molecule; CGA, chromogranin A; SYN, synaptophysin; NA, not assessed.

was not completely accepted at our hospital at that time.

The results of the immunohistochemical staining are shown in Table 2, and a typical case showing positive staining is shown in Fig. 1B and D. Of the 20 LCNECs, 19 expressed at least one of the three general neuroendocrine markers, namely CGA, SYN, and NCAM. Sixteen of the 20 LCNECs exhibited positive staining for NCAM, while one showed equivocal staining. Twelve of the 20 LCNECs showed positive staining for CGA. Thirteen LCNECs showed positive staining for SYN and three showed equivocal staining. Only one case was negative for all the three general neuroendocrine markers, however, this case exhibited the typical histological features of LCNEC on light microscopy.

The clinical characteristics of the patients are summarized in Table 1. The extremely high predominance of men and smokers in this study was comparable to the demographic features of our LCNEC patients treated by surgical resection [6]. Previous chemotherapy was given in six patients: nedaplatin in one and cyclophosphamide-based regimen in five patients. The chemotherapy regimens used were as follows: cisplatin (80 mg/m^2, day 1) and etoposide (100 mg/m^2, days 1–3) (n = 9), cisplatin (80 mg/m^2, day 1), vindesine (3 mg/m^2, days 1 and 8) and mitomycin (8 mg/m^2, day 1) (n = 6), cisplatin (80 mg/m^2, day 1) and vindesine (3 mg/m^2, days 1 and 8) (n = 4), or cisplatin (100 mg/m^2, day 1) alone (n = 1). The median (range) number chemotherapy cycles were 2 (1–6). Of the 20 patients, one showed CR and nine showed PR, yielding an overall response rate of 50% (95% confidence interval, 27.2–72.8%). One CR and four PRs were observed among the cases treated with cisplatin and etoposide, two PRs were found among those treated with cisplatin, vindesine and mitomycin, and three PRs were found among those treated with cisplatin and vindesine. Seven patients showed NC, and three showed progressive disease. While the response rate did not differ between patients with an initial diagnosis of SCLC and those patients with an initial diagnosis of NSCLC, previous chemotherapy affected the response to cisplatin: the response rate in chemo-naive patients was 64%, whereas that in previously treated patients was 17%. The median progression-free survival in the 20 patients was 103 days, median survival was 239 days, 1-year survival rate was 35%, and 2-year survival rate was 15%.

4. Discussion

In this extensive review of over 3000 lung cancer patients, we found considerable difficulty in evaluating the response of LCNEC to systemic chemotherapy. The pathological diagnosis of LCNEC was established in 87 (3.1%) of 2790 patients treated by surgical resection. This low incidence of LCNEC in surgically treated lung cancer patients is comparable to that in other previously published reports: 2.4% (50/2070), 2.9% (22/766), and 3.6% (53/1530) [16–18]. Of the 87 patients, only five who had received cisplatin-based chemotherapy for recurrent tumor that was evaluable for the response. While LCNEC is difficult to diagnose prior to the start of treatment on the basis of the findings in biopsy or cytological specimens, the architectural neuroendocrine features may, more or less, be reflected in these small samples [19,20]. We, therefore, conducted a review of 567 autopsy cases of lung cancer, and identified 15 cases of LCNEC who had received cisplatin-based chemotherapy. We obtained a response rate to cisplatin-based chemotherapy of 50% in these 20 patients with LCNEC, however, the clinical characteristics of patients with medically treatable advanced LCNEC would still remain to be clarified, because autopsy is conducted only in highly selective cases.

Travis et al. suggested that immunohistochemical or electron-microscopic evidence of neuroendocrine features were important to diagnose LCNEC [1]. We assessed the neuroendocrine marker expression by immunohistochemical staining for CGA, SYN, and NCAM. Our cases included one that was negative for all the three neuroendocrine markers examined, but showed the typical histological features of LCNEC, which could be attributable to technical staining problems. Immunohistochemical staining for neuroendocrine tumors is generally recognized as only a supplementary diagnostic tool. In addition, the post-surgical survival rate did not differ between histologically diagnosed cases of LCNEC with neuroendocrine differentiation in marker expression as assessed by immunohistochemical staining and large cell carcinoma with neuroendocrine morphology where the neuroendocrine markers were negative (data not shown). Thus, we decided to include the case with negative staining as LCNEC on the basis of its typical neuroendocrine morphology.

To the best of our knowledge, only one study on the efficacy of chemotherapy in patients with LCNEC has been reported previously. In the study, 13 patients with LCNEC received chemotherapy when relapse was noted after surgical resection, and two (20%) of 10 evaluable patients showed an objective response. The evaluable lesion in these patients, however, was the brain in seven, liver in two, and bone in one patient [21]. Thus, the relatively low response rate in the report may be due to the site of the evaluable lesion. In addition, reports on the correlation between response to chemotherapy and neuroendocrine differentiation of NSCLC may be helpful. Graziano et al. reported that the proportion of NSCLC positive for neuroendocrine markers was higher in responders than in non-responders among 52 NSCLC patients treated by chemotherapy, and that the result suggested a correlation between positivity for neuroendocrine marker expression and the likelihood of response to chemotherapy [7]. On the other hand, others have reported the absence of any correlation between the presence of neuroendocrine differentiation and the response to chemotherapy [8,9]. The neuroendocrine differentiation in NSCLCs in the aforementioned studies was confirmed only by immunohistochemical staining and not on the basis of the morphological definition of LCNEC. Therefore, these groups might have potentially included heterogeneous subtypes of lung carcinoma, such as adenocarcinoma or squamous cell carcinoma, with components of neuroendocrine differentiation. The conflicting conclusions of these studies may, therefore, reflect differences in the biological characteristics of the tumors included in the analysis. Since the definition of LCNEC is based on morphological criteria as well as positivity for neuroendocrine marker expression, LCNEC is may be considered to be a clinically homogeneous group. Therefore, our study of LCNEC may endorse the former reports about the relationship between neuroendocrine differentiation and the sensitivity to chemotherapy.

Objective response to chemotherapy can be observed in only 15–30% of NSCLCs, even when they are treated with regimens containing cisplatin [10]. In SCLC, however, effective combination regimens yield objective response rates in the range of 80–90% [11]. Our study showed an overall response rate of LCNEC of 50% to cisplatin-based chemotherapy, and a response rate of 64% in chemo-naive patients, which seemed to be higher than the response rate of NSCLC to chemotherapy. Considered together, these results suggest that the chemosensitivity of LCNEC is intermediate between that of NSCLC and SCLC, although we were unable to obtain firm evidence from this retrospective study, which included only a small cohort of patients.

Since LCNEC is a relatively rare subtype of lung cancer, a prospective study is difficult to perform, and may only be possible as a multicenter study.

For this purpose, it is an urgent task to establish diagnostic criteria for LCNEC based on examination of biopsy or cytologic specimens. Although the histological definition of LCNEC in surgically resected specimens proposed by Travis et al. is commonly accepted, its diagnostic reproducibility is not satisfactory [22]. It is also difficult to apply the definition to biopsy specimens, in which artifacts can easily be produced and detailed examination may be difficult due to insufficient specimen size. Thus, definitive diagnostic criteria also applicable to biopsy and cytologic specimens are required.

Our study did not include any cases labeled as LCNEC at the time of initial diagnosis. One half of the cases was originally diagnosed as SCLC and the other half as NSCLC, including poorly differentiated adenocarcinoma and large cell carcinoma. This was attributed to the fact that the concept of LCNEC was not clearly defined prior to its being proposed by Travis et al. [1]. Thus, it is possible that patients with LCNEC were included in earlier clinical trials for NSCLC or SCLC. If LCNEC shares the poor prognosis of NSCLC, the reported results of chemotherapy for NSCLC may have been worse in studies in which cases of LCNEC were included. Similarly, the results of clinical studies of SCLC to study their objective response to chemotherapy may also have been worse because of the confounding effects of the inclusion of LCNECs among the cases.

In conclusion, our results suggest that the response rate of LCNEC to cisplatin-based chemotherapy was comparable to that of SCLC. However, because of the retrospective nature of this study and the small sample size, we could not arrive at any definitive conclusion; we, therefore, propose to conduct a prospective study in the future aimed at elucidating the efficacy of chemotherapy for LCNEC. To that end, firm diagnostic criteria for LCNEC need to be established, even when the diagnosis must be based only on examination of biopsy and cytology specimens.

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Highly specific marker genes for detecting minimal gastric cancer cells in cytology negative peritoneal washings

Kazuhiko Mori,[a,f] Kazuhiko Aoyagi,[a] Tetsuya Ueda,[f] Inaho Danjoh,[b] Yasuhiro Tsubosa,[g] Kazuyoshi Yanagihara,[c] Yoshihiro Matsuno,[d] Mitsuru Sasako,[e] Hiromi Sakamoto,[a] Ken-ichi Mafune,[f] Michio Kaminishi,[f] Teruhiko Yoshida,[a,b] Masaaki Terada,[a] and Hiroki Sasaki[a,*]

[a] *Genetics Division, National Cancer Center Research Institute, Tokyo 104-0045, Japan*
[b] *Center of Medical Genomics, National Cancer Center Research Institute, Tokyo, Japan*
[c] *Central Animal Laboratory, National Cancer Center Research Institute, Tokyo, Japan*
[d] *Clinical Laboratory, National Cancer Center Hospital, Tokyo, Japan*
[e] *Surgical Oncology Department, National Cancer Center Hospital, Tokyo, Japan*
[f] *Department of Gastrointestinal Surgery, University of Tokyo Graduate School of Medicine, Tokyo, Japan*
[g] *Division of Esophageal Surgery, Shizuoka Cancer Center Hospital, Shizuoka, Japan*

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Abstract

Peritoneal wash cytology plays a pivotal role in the decision for gastric cancer treatment because advanced gastric cancer often turns out incurable with peritoneal metastasis. Molecular detection of minimal cancer cells from peritoneal washings may overcome the sensitivity boundary of conventional cytology and contribute to the prediction of the disease outcome. To select marker candidates out of ten thousands of genes, we performed microarray analyses in 12 gastric cell lines and 8 peritoneal washings of early stage cases. With 40 candidates selected by the above expression profiling, RT-PCR in 16 representative peritoneal wash samples was performed to identify genes specific to cytology positive samples. The finally selected five genes, *CK20*, *FABP1*, *MUC2*, *TFF1*, and *TFF2*, were then evaluated for their utility as a marker for minimal residual disease in 99 peritoneal wash samples. Nested RT-PCR using the five genes showed positive results highly specific to incurable cases (91–100%). With a high specificity, the combination of these five genes succeeded in identifying 6 out of 20 (30%) additional patients with all types of early recurrence that could not be predicted by the conventional method. The six newly identified recurrences included four non-peritoneal ones, showing that RT-PCR using the five genes without a real-time quantitative PCR technique contributes to the detection of minimal residual disease.

Keywords: Molecular marker; Gastric cancer; Peritoneal washing; Minimal residual disease

In the detection of cancer cells from various kinds of samples, many researchers aim for the development of new techniques that overcome the detection limit of conventional immunostaining and cytology. Methylation-specific PCR in ductal lavage fluid for breast cancer [1] and digital protein truncation assay in a stool sample for colorectal cancer [2] are good examples. mRNAs are also a good target for cancer cell detection. To date, many reports on mRNA detection of cancer cells have been published but the essential task, the selection of candidate genes, is knowledge-dependent in most of those previous studies.

As for gastric cancer, mRNAs from peritoneal washings or lymph nodes are used to demonstrate minimal residual disease in investigational studies [3–5]. For advanced gastric cancer, peritoneal cytology plays the pivotal role in the decision for treatment strategy because peritoneal metastasis is the commonest mode of recurrence [6] and discourages radical surgery. However,

* Corresponding author. Fax: +81-3-3541-2685.
E-mail address: hksasaki@gan2.res.ncc.go.jp (H. Sasaki).

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not a few gastric cancer patients with negative cytology develop peritoneal metastasis as a result of the sensitivity boundary of cytology. mRNA detection of cancer cells from peritoneal washings may provide us with a more sensitive way, and efforts are being made to establish detection techniques. Carcinoembryonic antigen (*CEA*) is a well-known tumor marker and has been used to detect a very small number of adenocarcinoma cells in the blood, peritoneal wash or other body fluids [3,7,8]. The expression of *CEA* mRNA, however, is not specific to cancer cells and often produces false positive results [9]. Other more specific genes are awaited for more accurate diagnosis.

Therefore, we conducted a gene screening using microarray in a search for a molecular marker of gastric cancer cells in the peritoneal cavity.

Materials and methods

Peritoneal wash samples and patient profiles. One hundred and eight peritoneal wash samples and 46 gastric cancer tissues were collected from 108 out of 235 consecutive gastric cancer patients who gave written informed consent and whose surgery was performed from August 1999 to March 2000 at the National Cancer Center Hospital, Japan. Patients with clinically advanced disease were always candidates for peritoneal wash cytology. One hundred milliliters of the peritoneal washings collected during surgery was halved for cytology and RNA extraction. If massive ascites was present, the ascites was sampled instead. From 21 patients with early disease, the entire wash samples were used only for RNA extraction. Nine patients were excluded because of loss of follow-up, recent history of another intra-abdominal cancer, or metachronous peritoneal metastasis after positive surgical margin. All disease-free patients were followed up at least 2 years. Patients were evaluated for recurrence through consultation visit, measurement of serum CEA, and CA19-9 at 3-month intervals, and clinical imaging once a year including chest X-ray, abdominal ultrasonography, and computerized tomography scanning. In cases where recurrence was suspected, these routine imaging techniques and barium contrast enema were used for the establishment of diagnosis. Patients without evident incurable factors had no adjuvant therapies as long as no disease recurrence was confirmed except for a small number of participants in a clinical trial.

Cell lines. As a positive control for the first gene screening with microarray, we used gastric cancer cell lines instead of positive cytology samples because the cellular population of cancer cells on cytology positive slides was below 10% in most cases. Eight cell lines derived from diffuse-type gastric cancer, HSC39, HSC43, HSC44, HCS58, HSC59, HSC60, OCUM2M, and KATO III, and four cell lines from intestinal-type gastric cancer, HSC57, MKN7, MKN28, and MKN74,

Table 1
Primer sequences

Gene	Outer forward primers Outer reverse primers	Inner forward primers Inner reverse primers
CK20	5′-CAGACACACGGTGAACTATGG-3′ 5′-GATCAGCTTCCACTGTTAGACG-3′	5′-GGGACCTGTTTGTTGGCAATG-3′ 5′-ATTTGCAGGACACACCGAGCAT-3′
FABP1	5′-GAGCCAGGAAAACTTTGAAGC-3′ 5′-CAATGTCACCCAATGTCATGG-3′	5′-TCATGAAGGCAATCGGTCTG-3′ 5′-GTGATTATGTCGCCGTTGAGT-3′
MUC2	5′-CCGGGGAGTGCTGTAAGAAG-3′ 5′-GCTCTCGATGTGGGTGTAGG-3′	5′-GACAACCAGCACGTCATCCT-3′ 5′-CTCCTCTTTGCAGCAGGAGC-3′
TFF1	5′-CAGACAGAGACGTGTACAGT-3′ 5′-AAGTCAGAGCAGTCAATCTGT-3′	5′-CCCGTGAAAGACAGAATTGTG-3′ 5′-CCGAGCT-CTGGGACTAATCA-3′
TFF2	5′-CTGGAATCACCAGTGACCAGT-3′ 5′-GGCACTTCAAAGATGAAGTTG-3′	5′-ATGGATGCTGTTTCGACTCC-3′ 5′-GGCACTTCAAAGATGAAGTTG-3′
MASPIN	5′-CTCACAATAGCCGATATCAGA-3′ 5′-AATCTCAGAACAAGAAGAACCT-3′	
GW112	5′-CTCCTCGAGGGACCAAATCT-3′ 5′-CACTTTGTCACTGCCATCAG-3′	
PRSS4	5′-CTGGGCACAGTTGCTGTCCC-3′ 5′-GGCCACCAGAGTCACGCTGG-3′	
MDK	5′-CCTGCAACTGGAAGAAGGAG-3′ 5′-GGAGGCTCAAGCTTCCCAGA-3′	
SOX9	5′-GGTTGTTGGAGCTTTCCTCA-3′ 5′-AGCAATCCTCAAACTCTCTAG-3′	
CDX1	5′-ACTGAACGGCAGGTGAAGAT-3′ 5′-AGGGTGGATAGGTGACTGTC-3′	
CEA	5′-TCTGGAACTTCTCCTGGTCTCTCAGCTGG-3′ 5′-TGTAGCTGTTGCAAATGCTTTAAGGAAGAAGC-3′	5′-GGGCCACTGTCGGCATCATGATTGG-3′ 5′-TGTAGCTGTTGCAAATGCTTTAAGGAAGAAGC-3′
β-Actin	CACTGTGTTGGCGTACAGGT-3′ TCATCACCATTGGCAATGAG-3′	

were maintained in RPMI1640 supplemented with 10% fetal calf serum, 0.15% sodium bicarbonate, 2 mM L-glutamine, and penicillin–streptomycin. Cells were harvested only for RNA extraction.

RNA extraction. All the specimens and the pellets of the peritoneal washings were frozen immediately in liquid nitrogen and stored at −80 °C until use. For total RNA isolation, ISOGEN kit (Nippon Gene, Toyama, Japan) was used.

Gene screening strategy using microarray. We performed microarray analyses of gene expression in the clinical materials and gastric cell lines to screen out marker genes capable of detecting gastric cancer cells in the peritoneal washings using Affimetrix U95Av2 GeneChips (Affymetrix, Santa Clara, CA) featuring 12,625 transcripts. The procedures were conducted according to the supplier's protocols as described previously [10]. The arrays were scanned using the GeneArray scanner (Affymetrix) at 3-μm resolution, and the scanned image was analyzed quantitatively with the computer software Microarray Suite 4.0 (Affymetrix) to generate raw signal intensity data. In order to compare mRNA expression levels among samples, we normalized the raw data by setting the mean of the signal intensities of all probe sets to 1000 in each sample. The normalized signal intensity is referred to as AD (average difference) score. For statistical analysis to select genes, Microsoft Excel was used. First, microarray analyses were performed in 8 peritoneal wash samples from early gastric cancer patients and 12 gastric cancer cell lines. The eight peritoneal washings were used as negative control and the 12 cell lines as positive control to find genes specific to cancer cells. Seventy genes were extracted and then 46 gastric cancer tissues were also analyzed by microarray to exclude genes rarely expressed in primary gastric cancers. The finally selected genes were then validated by reverse transcription (RT)-PCR analyses in peritoneal washings from patients whose clinical outcome was followed up at least 2 years.

RT-PCR analysis in RNA samples from peritoneal washings. To overcome the limitation in the amount of samples needed for frequent RT-PCR analyses, we produced hundreds of micrograms of cDNA from 1 to 5 μg total RNA prepared from the peritoneal washings of 104 gastric cancer patients by an efficient method of high-fidelity mRNA amplification, called TALPAT [10]. RT-PCR was carried out in a total volume of 50 μl containing upstream and downstream primers (0.1 nmol each), 2.0 mM $MgCl_2$, 0.25 mM dNTP, 0.1 μg of the cDNA from each patient, and 2.5 U Ex *Taq* polymerase (Takara, Shiga, Japan).

The first PCR was cycled 25 times and another 15 cycles with 1/20 μl from the first PCR solution was added for nested PCR. For *CEA*, each cycle was heated at 95 °C for 1 min and 72 °C for 2.5 min, and for all of the other genes, 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. PCR was cycled the above times followed by incubation at 72 °C for 10 min. The PCR products were separated by electrophoresis in 2%

Table 2
Frequency of expression in primary cancers

	All types ($n = 46$)	Intestinal type[a] ($n = 17$)	Diffuse type[b] ($n = 29$)	P value[c]
CK20	20 (43.5)	8 (47.1)	12 (41.4)	0.71
FABP1	25 (54.3)	11 (64.7)	14 (48.3)	0.28
MUC2	17 (37.0)	7 (41.2)	10 (34.5)	0.65
TFF1	45 (97.8)	16 (94.1)	29 (100)	0.19
TFF2	35 (76.1)	11 (64.7)	24 (82.8)	0.17
MASPIN	32 (69.6)	15 (88.2)	17 (58.6)	0.04
GW112	36 (78.3)	14 (82.4)	22 (75.9)	0.61
PRSS4	41 (89.1)	17 (100)	24 (82.8)	0.07
MDK	40 (87.0)	15 (88.2)	25 (86.2)	0.84
SOX9	43 (93.5)	17 (100)	26 (89.7)	0.17
CDX1	19 (41.3)	9 (52.9)	10 (34.5)	0.22

[a] Intestinal type includes well and moderately differentiated adenocarcinoma.

[b] Diffuse type includes poorly differentiated adenocarcinoma and signet ring cell carcinoma.

[c] The proportional differences were calculated by χ^2 test.

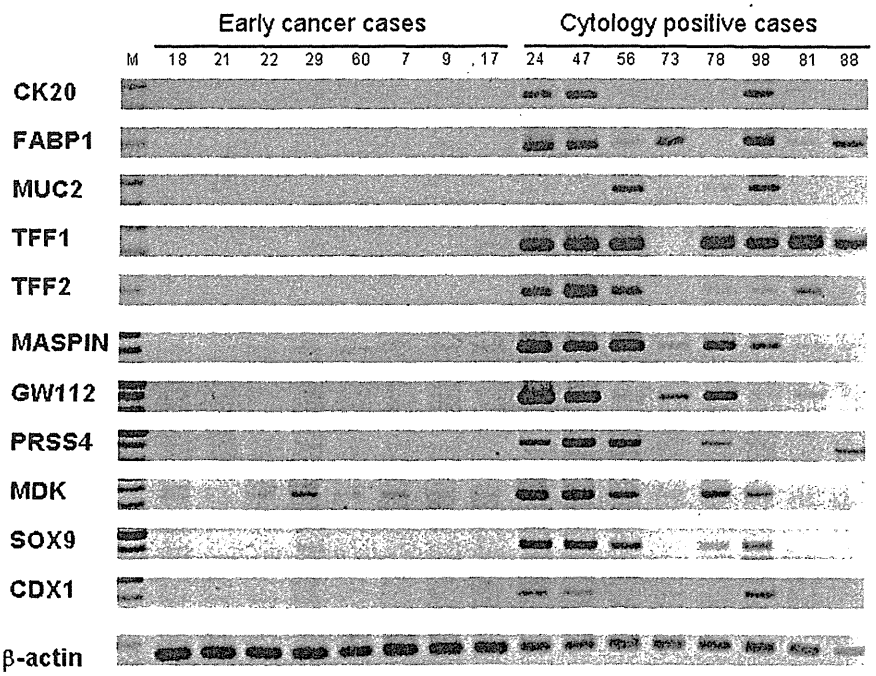

Fig. 1. Results of RT-PCR on peritoneal washings. The mRNAs of five genes, *CK20*, *FABP1*, *MUC2*, *TFF1*, and *TFF2*, were detected in most of the cytology-positive cases, but in none (except for TFF1) of the eight early gastric cancer cases even after a nested PCR. The other genes, *MASPIN*, *GW112*, *PRSS4*, *MDK*, *SOX9*, and *CDX1*, also showed correct results by a single PCR. The integrity of extracted RNA was confirmed by RT-PCR of *β-actin* mRNA. Top of each lane: M, a size marker, and each number, a sample No.

agarose gel, stained with ethidium bromide, and visualized under ultraviolet transilluminator. Primer sequences are shown in Table 1.

Statistical analyses. Survival analyses were made by Kaplan–Meier curves with the diagnosis of disease recurrence as the endpoints.

Results

The peritoneal washings from gastric cancer patients contain erythrocytes, leukocytes, dissociated peritoneal mesothelium, and a small number of cancer cells. Therefore, genes, which express in cancer cells but not in leukocytes or the mesothelium, are considered to be targets for detecting minimal gastric cancer cells in peritoneal washings. The first step of gene selection using a microarray of 12,625 transcripts left 70 genes whose mRNAs were not only undetectable (AD score corresponding to a signal intensity is below 500) in the peritoneal washes of all the eight early gastric cancer patients, but also abundant (AD score higher than 5000) in at least one of the 12 gastric cell lines. *CEA* did not fulfill this requirement because the AD score exceeded the threshold of 500 in only one peritoneal wash sample. In the second step, 40 genes out of the 70 were selected considering their known functions, tissue specificity, and expression levels in the primary gastric cancer tissues. The final step for the 40 genes, in which 16 representative peritoneal wash samples derived from both eight early cases and eight cytology-positive cases were analyzed by RT-PCR, yielded 11 genes whose mRNAs were detected in the cytology-positive cases but not in the early gastric cancers. Among them, mRNAs of five genes, *TFF1* (except for No. 17), *TFF2*, *FABP1*, *CK20*,

Table 3
Cytology and RT-PCR results in 99 patients

Cytology	Incurable disease (*n*)	CK20	FABP1	MUC2	TFF1	TFF2	Two or more of five genes	CEA
Positive (18)	Peritoneal	10	12	7	15	13	15	17
Negative[a]	None[b] (55)	0	3	0	5	4	1	10
	Peritoneal (9)	4	4	3	3	2	4	7
	Non-peritoneal metastasis (13) (recurrence)	2	5	1	7	4	4	7
	Proportional differences[c]	<0.04	0.005	0.19	0.0009	<0.04	0.004	0.013
	Non-peritoneal metastasis (3) (synchronous)	0	0	0	0	1	0	1
	Unresectable (1)	1	0	0	0	0	0	1
Overall specificity to incurable cases (%)		100	95	100	91	93	98[b]	82
Sensitivity to cytology positive cases (%)		56	67	39	83	72	83	94

[a] Including clinically early stage cases for which cytology was not performed.
[b] Two cases for TFF1 and CEA and one for TFF2 developed recurrence after 2 years. The only case positive for more than two markers also had peritoneal recurrence and, accordingly, the specificity to incurable cases was 100% finally.
[c] Proportional differences between non-peritoneal recurrence and recurrence free group. One-sided Fisher's exact test.

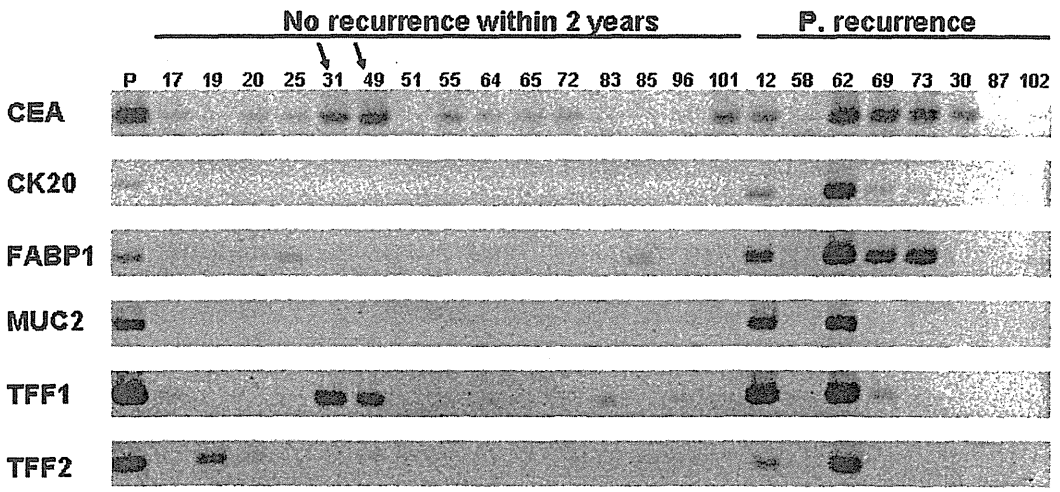

Fig. 2. Representative RT-PCR results of the five type A genes and *CEA* in peritoneal washings of 99 gastric cancer patients. Ten positive cases for *CEA* (18%) were observed in the 55 patients who had no incurable factor and did not develop recurrence within 2 years, while there were no positive cases for *CK20* and *MUC2* (0%), 3 for *FABP1* (5%), 4 for *TFF2* (7%), and 5 for *TFF1* (9%). Two cases (No. 31 and 49) eventually had recurrence after more than 900 days (arrow).

and *MUC2* were not detectable in the eight early cases even after a nested PCR (Fig. 1). The other genes, *MASPIN*, *GW112*, *PRSS4*, *MDK*, *SOX9*, and *CDX1*, also showed correct results by a single PCR but produced positive bands in the early cases by nested PCR. The frequency of their expression in the 46 gastric cancer tissues by microarray analyses is shown in Table 2. Almost none of these 11 genes had a significant difference in their frequency between intestinal-type and diffuse-type cancers, suggesting that these genes can be markers detecting gastric cancer cells derived from either major type of gastric cancer.

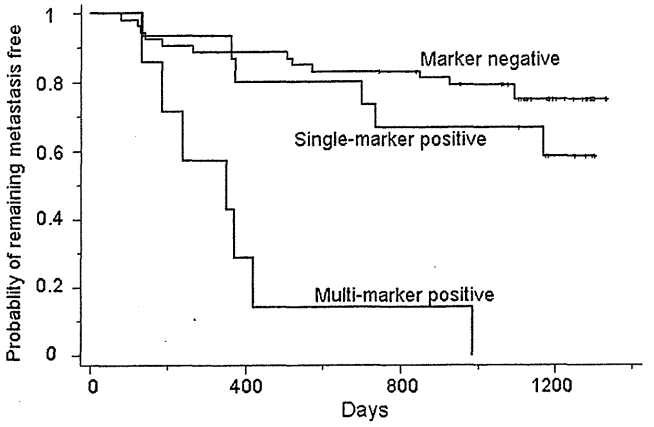

Fig. 3. Disease outcome of 75 potentially curative patients without synchronous metastases or positive cytology. Probability of metastases was significantly different among groups classified according to the result of RT-PCR with the five markers identified in this study (log-rank test, $P < 0.0001$).

Next, expression of the former five genes together with *CEA* was examined by nested PCR in the 99 peritoneal wash samples. Table 3 shows the number of positive results in each group of patients divided according to their cytology and disease outcome. Fifty-five patients were free from disease recurrence within 2 years after surgery and positive results in this group were 10 for *CEA* and 0–5 for the five genes identified in this study. Thus the specificity of the 5 genes identified in this study to early recurrence was 91–100%. Multiple (two or more) positive results with the five genes (not including *CEA*) were far more reliable and produced better specificity and sensitivity and, therefore, may be defined as high-risk of recurrence. The specificity may be improved with further followup and, actually, two additional cases

Table 4
Clinical outcome and disease stage of 24 patients with two or more positive RT-PCR results

Cases	Tumor depth	Nodal metastases[a]	Main metastatic site	DFS[b]	CK20	FABP1	MUC2	TFF1	TFF2
1	T2	10/45	Peritoneum	986	−	−	−	+	+
2	T3	16/84	Peritoneum	239	+	+	−	+	−
3	T3	19/75	Peritoneum	348	+	+	+	+	+
4	T2	9/73	Liver	183	−	+	−	+	−
5	T2	41/64	Liver	369	−	+	−	+	+
6	T3	9/78	Lung	418	+	+	−	+	+
7	T3	27/70	Systemic[c]	130	+	+	+	+	+
				CY,[d] P					
8	T3	28/37	Lymph nodes[e]	CY+, P−	−	−	−	+	+
9	T3	46/59	Peritoneum	CY+, P−	−	+	−	+	+
10	T3	27/59	Peritoneum	CY+, P−	−	+	−	+	+
11	T3	5/84	Peritoneum	CY+, P−	+	+	−	+	−
12	T4	22/33	Peritoneum	CY+, P−	+	+	−	+	+
13	T3	50/84	Systemic[c]	CY+, P−	+	−	+	+	+
14	T3	9/48	Peritoneum	CY+, P+	+	+	−	−	−
15	T3	16/47	Peritoneum	CY+, P+	−	+	−	+	+
16	T4	23/51	Peritoneum	CY+, P+	−	−	+	+	+
17	T3	0/4	Peritoneum	CY+, P+	+	+	−	+	+
18	T4	—	Peritoneum	CY+, P+	+	+	+	+	+
19	T4	—	Peritoneum	CY+, P+	+	+	+	+	+
20	T3	11/44	Peritoneum	CY+, P+	+	+	+	+	+
21	T3	—	Peritoneum	CY+, P+	+	+	+	+	+
22	T3	18/21	Peritoneum	CY+, P+	+	+	+	+	+
23	T4	25/62	Peritoneum	CY−, P+	+	+	+	−	−
24	T3	18/40	Peritoneum	CY−, P+	+	+	+	+	+

[a] Metastatic/investigated nodes.
[b] Disease free survival (days).
[c] Multifocal hematogenous metastasis was observed but no evident peritoneial disease.
[d] Cytology and macroscopic peritoneal nodules.
[e] Obstructive jaundice due to metastatic nodes in hepatoduodenal membrane.

of recurrence were observed in the RT-PCR positive cases after more than 900 days after surgery (Table 3 and Fig. 2). The impact of these markers on the disease outcome after potentially curative surgery was shown by the Kaplan–Meier curves and log-rank test (Fig. 3). Patients with multiple-positive results with these five genes had a significantly poorer prognosis than the rest of the patients.

Interestingly, a notably high proportion of positive results was observed in the non-peritoneal recurrence group (Table 3, one sided Fisher's exact test, $p = 0.19$–0.0009). Table 4 shows all cases with multiple positive results and the pathological stage and disease outcome of such cases. Most cases had evident or retrospectively proven peritoneal free cancer cells but four hematogenous recurrences were observed among them. It may be safely presumed that these four patients initially had minimal residual disease in their peritoneal cavity that could have predicted disease recurrence. RT-PCR with the combination of five genes was able to detect 6 out of 20 additional cases of early recurrence that the conventional method was unable to predict. Moreover, out of 29 incurable cases without synchronous macroscopic incurable factor (such as peritoneal nodules and/or liver metastasis), this method alone could predict 12 cases whereas conventional cytology alone identified nine cases.

Fig. 2 shows all RT-PCR positive results for patients without early recurrence and with peritoneal recurrence after negative cytology. Cases 31 and 49 eventually developed peritoneal recurrence (disease free survival = 986 days) and Krukenberg Tumor (disease free survival = 1167), respectively, after a long occult disease stage period. The rest of the positive bands in the no-recurrence group were generally very weak.

Discussion

Microarray technique has been reported to have a great potential for identifying genes able to classify phenotypically identical tumors [11,12] or predict tumor sensitivity to chemotherapeutic or hormonal agents [13,14]. Our present study demonstrated that this technique is also a very useful tool for identifying molecular markers for detecting minimal residual disease.

Other analyses directly comparing the gene expression of peritoneal washings from eight early gastric cancer and four cytology positive cases yielded a set of inflammation-associated genes; however, RT-PCR analyses on these genes including *GRO3*, *SCYA20*, and *PTSG2* failed on identification of marker genes specific to cytology positive samples.

FABP1/L-FABP encodes the liver fatty acid binding protein and is expressed only in the epithelial absorptive cells of the duodenum, jejunum, ileum, and colon, but not in the esophagus and stomach, while its mRNA expression in gastric cancer has not been reported yet [15]. Accordingly, our data are the first to show the frequent expression of *FABP1* in gastric cancers. CK 7 and CK 20 are reportedly potentially useful markers for an immunohistochemical study to rule out patients with a high risk of Barrett's esophagus and cancer [16], and CK20 for the detection of lymph node micrometastases in patients with gastric cancer [5]. A subset of gastric cancers has been reported to express *TFF1*, *TFF2*, and *MUC2* mRNAs [17].

It is quite interesting that 13 patients with non-peritoneal recurrences in the potentially curative patients frequently showed positive results by RT-PCR. It is a generally accepted concept that the tumor cells dissociated from the primary lesions do not always form metastatic lesions [18]. To form a peritoneal metastasis, tumor cells need to survive after the dissociation from the primary lesion. In gastric cancer, the expression of integrin $\alpha 6\beta 4$ was shown to induce apoptosis of gastric cancer cell lines injected into mouse peritoneal cavity and that its expression in gastric cancer inversely correlated with the frequency of peritoneal metastases [19]. In the cases that developed non-peritoneal recurrence, we may have detected gastric cancer cells that could not survive in the peritoneal cavity long enough to form peritoneal metastases. The detection of free cancer cells in the peritoneal cavity does not necessarily predict peritoneal recurrence but may well be interpreted as their malignant potential characterized by the resistance to apoptosis induced by the dissociation from the primary lesion.

The present results demonstrate that nested RT-PCR on the peritoneal washings using multiple genes is a specific and sensitive tool for detecting rare gastric cancer cells in the peritoneal cavity, so that gastric cancer patients are able to receive more suitable individualized therapies. Benefits from adjuvant therapies or radical surgery are still a matter of controversy [20–23]. However, most past clinical trials were designed for non-stratified patients and thus may have buried the potential benefit of adjuvant therapies confined to the very early stage of metastatic disease. Patients with minimal residual disease after sufficient local control may be the most eligible patients for clinical trials of post-operative adjuvant chemotherapy. After confirmation of our findings, future clinical trials featuring the molecular markers identified in this study may be of great importance in finding out more suitable treatment strategies for gastric cancer patients.

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CT Findings of Surgically Resected Large Cell Neuroendocrine Carcinoma of the Lung in 38 Patients

Yasuji Oshiro[1]
Masahiko Kusumoto[2]
Yoshihiro Matsuno[3]
Hisao Asamura[4]
Ryousuke Tsuchiya[4]
Hiroshi Terasaki[5]
Hidefumi Takei[3]
Arafumi Maeshima[6]
Sadayuki Murayama[1]
Noriyuki Moriyama[2]

OBJECTIVE. We sought to assess the CT features of surgically resected large cell neuroendocrine carcinoma of the lung.

MATERIALS AND METHODS. The cases of all patients who underwent surgical resection for primary lung cancer in a single institution from 1993 to 2000 and who received an initial diagnosis of poorly differentiated non–small cell lung carcinoma, small cell carcinoma, carcinoid tumor, and large cell neuroendocrine carcinoma were histologically reviewed. The findings for 43 patients were histologically reclassified and confirmed as large cell neuroendocrine carcinoma. The CT scans available for 38 patients were evaluated by two observers.

RESULTS. In the 38 patients, six central tumors and 32 peripheral tumors, with diameters ranging from 12 to 92 mm (mean ± SD, 32 ± 19 mm), were identified. None of the tumors had air bronchograms or calcification in the mass or nodule. Of the 19 patients with thin-section CT scans, 14 (74%) showed the tumor–lung interface as well defined and five (26%) showed the interface to be ill defined. Lobulation was identified on 15 scans (79%) and spiculation was evident on six scans (32%). On contrast-enhanced CT scans, inhomogeneously enhanced tumors appeared to be larger (51 ± 18 mm) than homogeneously enhanced tumors (25 ± 10 mm; $p < 0.001$). At histopathologic examination, gross necrosis was noted in 20 of 28 patients who had undergone contrast-enhanced CT, and the cause of inhomogeneous enhancement on CT scans was determined to be intratumoral necrosis. Multiple microscopic necroses were present in all 28 patients.

CONCLUSION. Large cell neuroendocrine carcinoma usually appears as a well-defined and lobulated tumor with no air bronchograms or calcification. The inhomogeneous enhancement (caused by necrosis) seen in large cell neuroendocrine carcinomas with large diameters is not necessarily apparent in small-diameter (< 33 mm) large cell neuroendocrine carcinomas, even if the tumor contains necrosis.

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[1]Department of Radiology, University of Ryukyus School of Medicine, 207 Uehara, Nishihara-cho, Okinawa Prefecture 903-0215, Japan.

[2]Diagnostic Radiology Division, National Cancer Center Hospital, 1-1 Tsukiji 5-chome, Chuou-ku, Tokyo 104-0045, Japan. Address correspondence to M. Kusumoto.

[3]Clinical Laboratory Division, National Cancer Center Hospital, Tokyo 104-0045, Japan.

[4]Thoracic Surgery Division, National Cancer Center Hospital, Tokyo 104-0045, Japan.

[5]Department of Radiology, Kurume University School of Medicine, 67-Asahimachi, Kurume 830-0011, Japan.

[6]Pathology Division, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuou-ku, Tokyo 104-0045, Japan.

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In the revised World Health Organization (WHO) classification of lung cancer published in 1999, large cell neuroendocrine carcinoma was categorized as a variant of large cell carcinoma [1]. Large cell neuroendocrine carcinoma of the lung is defined as a poorly differentiated and high-grade neuroendocrine tumor that morphologically and biologically may be placed between atypical carcinoid tumor and small cell carcinoma [2]. Some researchers have reported that survival rates for patients with large cell neuroendocrine carcinoma are lower than those for patients with comparable stage I non–small cell lung carcinoma [3] or classic large cell carcinoma [4] and are no different than those for patients with small cell carcinoma [5]. Large cell neuroendocrine carcinomas of the lung have been more frequently studied in recent years; however, the number of patients with large cell neuroendocrine carcinoma is still relatively small in comparison to the numbers of patients with squamous cell carcinoma, adenocarcinoma, or small cell carcinoma. Several reports have described the pathologic and clinical features of large cell neuroendocrine carcinomas, but information on the imaging characteristics of these carcinomas is limited [6, 7]. For this reason, we analyzed the CT findings of 38 patients with resected large cell neuroendocrine carcinomas to determine the CT characteristics of these tumors and to identify any specific imaging features that may help in the diagnosis of the disease.

Materials and Methods

Between 1982 and 1999, 2,790 patients underwent surgical resection for primary lung carcinoma at the National Cancer Center Hospital. To

extract cases of large cell neuroendocrine carcinoma from this large pool of patients, we reviewed the records of 572 patients who had received one of the following histologic diagnoses: small cell carcinoma, poorly differentiated adenocarcinoma, poorly differentiated squamous cell carcinoma, large cell carcinoma, carcinoid tumor, and large cell neuroendocrine carcinoma. Three pathologists independently reviewed each case, resolving discrepancies among their findings by consensus. Immunohistochemical tests were performed on retained specimens to confirm the neuroendocrine phenotype in every patient. The details of histopathologic review of the cases have been described previously [3].

In 87 of the 572 patients, the carcinomas were reclassified and confirmed as large cell neuroendocrine carcinomas. CT scans were available for review in 34 of the 38 patients who underwent surgery during the most recent 7 years of the study period (1993–1999). In addition, we identified four patients with a histologically determined diagnosis of large cell neuroendocrine carcinoma who had undergone CT in 2000. Therefore, our study population consisted of 38 patients, 36 men and two women, whose ages ranged from 45 to 82 years (mean, 66 years). Of the 38 patients, 20 had lesions classified as large cell neuroendocrine carcinomas at the initial diagnosis. The initial pathologic diagnoses of the lesions in the remaining 18 patients were as follows: eight small cell carcinomas (six intermediate cell type), seven poorly differentiated adenocarcinomas, two large cell carcinomas, and one poorly differentiated squamous cell carcinoma. Thirty-one of 38 patients who presented without pulmonary symptoms had abnormal findings on chest radiographs obtained during a routine examination. Symptoms in the remaining seven patients included low-grade fever ($n = 1$), chest and back pain ($n = 1$), hemosputum ($n = 3$), and cough ($n = 2$). Thirty-one patients had been smokers for between 12 and 200 pack-years (mean, 62 pack-years). Six patients had quit smoking 3–17 years (mean, 7 years) before diagnosis. In one patient, a smoking history could not be obtained. Surgical procedures included a partial resection of the mass in four patients, segmentectomy in one patient, lobectomy in 27 patients, and pneumonectomy with regional lymph node dissection in six patients.

TABLE 1 CT Findings in 38 Patients with Surgically Resected Large-Cell Neuroendocrine Carcinomas

Finding	Patients	
	No.	%
Tumor location in lobe		
Peripheral	32	84
Central	6	16
Tumor location by lobe		
Right		
Upper	12	32
Middle	3	8
Lower	8	21
Left		
Upper	12	32
Middle	0	0
Lower	3	8
Internal characteristics		
Air bronchogram	0	0
Calcification	0	0
Cavity	1	3
Other findings		
Endobronchial growth	2	5
Obstructive pneumonia	3	8
Pleural effusion	9	24
Tumor–lung interface features[a]		
Well-defined	14	74
Ill-defined	5	26
Smooth	1	5
Lobulated	15	79
Spiculated	6	32
Ground-glass opacity	3	16
Surrounding emphysema[a]	4	21

Note.—*Average tumor size ± SD = 32 ± 19 mm.*

[a]These findings apply only to the 19 patients who underwent thin-section CT.

CT scans were obtained with X-Vigor or TCT-900S units (Toshiba, Tokyo, Japan). The helical technique (collimation, 10 mm; pitch, 1.5) was used and covered the area from the lung apices to the diaphragm. In 28 patients, nonionic iodinated contrast material (Iopamiron [iopamidol 300 mg I/mL], Nihon Schering, Osaka, Japan) was administered IV at a rate of 1–2 mL/sec. Contrast-enhanced CT scans were obtained within 120 sec (range, 70–120 sec) after the onset of contrast material infusion. Unenhanced scans were obtained in 20 of 28 patients who underwent contrast-enhanced CT. In 19 of 38 patients, additional thin-section (collimation, 2.0 mm; pitch, 1.0) scans were obtained at the level of the lesion. All CT scans were obtained with 120 kVp and 200 mA. The scans were viewed on standard mediastinal window setting (window level, 60 H; window width, 550 H) and lung window setting (window level, –600 H; window width, 1500–2000 H). The CT scans were assessed by two radiologists who reached conclusions by consensus.

CT scans were reviewed for location, size, and internal characteristics of the tumor. The descriptions of tumor location included identification of the affected lung lobe and of the position—central versus peripheral—of the tumor in the lobe. Central tumors were defined as those that involved the carina or a main segmental bronchus. Peripheral tumors were defined as those surrounded by lung parenchyma or distal to the subsegmental bronchi. The scans were evaluated for the presence of endobronchial growth, obstructive pneumonia or atelectasis, and pleural effusion. On thin-section CT, tumor–lung interface characteristics were assessed and the presence or absence of surrounding emphysema was noted.

Enhancement of the nodules was assessed subjectively by comparing the enhancement of the chest wall muscle on the unenhanced scans with corresponding enhancement on the contrast-enhanced scans. The enhancement patterns of the nodules were noted as either homogeneous or inhomogeneous. Inhomogeneity was defined as the presence of an area of low attenuation in the highly attenuated background of the nodule. Nodules with peripheral rim enhancement were also considered to have inhomogeneous enhancement. For the 28 patients who underwent contrast-enhanced CT, we reviewed the pathologic reports to identify those patients whose reports mentioned that necrosis was present in gross specimens and then correlated the presence of necrosis with the CT findings. Statistical analyses of size and macroscopic necrosis between homogeneously and inhomogeneously enhanced tumors were performed using Student's t test and Fisher's exact test, respectively. Mediastinal or hilar lymph nodes that were larger than 1 cm in the short-axis diameter were regarded as evidence of lymphadenopathy. Tumors were staged according to the TNM classification scheme [8]. No patients had extrathoracic metastases at the time of diagnosis. Distribution of the clinical stages was as follows: stage IA ($n = 20$); stage IB ($n = 7$); stage IIA ($n = 1$); stage IIB ($n = 3$); stage IIIA ($n = 5$); and stage IIIB ($n = 2$). The CT findings regarding the tumor–lung interface and intratumoral necrosis were correlated with findings at surgery and pathologic examination.

Results

The CT findings of large cell neuroendocrine carcinomas are summarized in Table 1. The right lung was involved in 23 (61%) and the upper lobes were involved in 24 (63%) of the 38 patients studied. The tumors were seen peripherally in 32 patients (84%) and centrally in six patients (16%). Endobronchial growth and obstructive pneumonia were associated only in patients with centrally located tumors. Neither air bronchograms nor calcification was observed in any patient.

Among the 19 patients who underwent thin-section CT, the more common tumor–lung interface characteristics were a well-defined interface (74%) and lobulation (79%) (Fig. 1). Spiculation (Figs. 2 and 3) was observed in six patients (32%). In four patients, however, spiculated margins appeared as fibrotic strands because of paracicatricial emphysema or linear opacities made more pronounced by preexisting emphysema (Fig. 2).

On contrast-enhanced CT, attenuation of all the tumors varied from slightly less than to more than the attentuation of the chest wall muscle. Among the 28 patients who had contrast-enhanced CT, 15 (54%) had tumors with

homogeneous enhancement (Figs. 2 and 3), and 13 tumors (46%) showed inhomogeneous enhancement. Of the inhomogeneously enhanced tumors, five had peripheral rim enhancement. As shown in Table 2, the inhomogeneously enhanced tumors were generally larger than the homogeneously enhanced tumors. At gross pathology examination of the resected tumors, necrosis was identified in seven of the 15 homogeneously enhanced carcinomas and in all of the 13 inhomogeneously enhanced carcinomas (Table 2). The largest homogeneously enhanced tumor with necrosis was 33 mm.

At histopathologic examination, pulmonary alveoli were filled entirely with tumor cells or had a compressive growth pattern, giving the appearance of a well-defined margin. Lobulated margins observed on CT reflected the interruption of tumor growth by anatomic structures or tumor extension due to differential tumor growth. In four patients, spiculation was attributable to paracicatricial or preexisting emphysema. In all other patients, spiculation corresponded to vascular and lymphatic invasion, a normal vascular structure, a thickened interstitium, or an irregularly protuberant tumor nest. In two patients, tumors associated with the bronchial wall were identified as endobronchial polypoid lesions that invaded the surrounding lung parenchyma. In one of the two patients, the invading lesion was accompanied by obstructive pneumonia. The cause of inhomogeneous enhancement was intratumoral necrosis.

Necrosis was noted in the gross pathology specimens of 20 of 28 patients who had contrast-enhanced CT scans. The difference between homogeneously and inhomogeneously enhanced tumors in the incidence of macroscopic necrosis was statistically significant ($p < 0.003$). Although the gross necrotic area was difficult to determine in the cut surface of the small tumors, multiple punctate necroses were observed microscopically in each case. In the large tumors, necrotic foci were larger and tended to be confluent. Consequently, the tumors contained extensive necroses. Inhomogeneous enhancement with peripheral rim enhancement represented a large central necrosis.

Discussion

The classification of pulmonary neuroendocrine tumors of the lung is complex and potentially confusing. For many years, carcinoid tumors and small cell lung carcinomas were the only two recognized types of these tumors. In 1972, Arrigoni et al. [9] proposed that bronchial carcinoid tumors be separated into typical and atypical variants, with the latter having more malignant histologic characteristics and clinical manifestations. Since then, neuroendocrine tumors of the lung have been frequently classified into three categories: typical carcinoid, atypical carcinoid, and small cell carcinoma [2, 10]. Most tumors classified morphologically as being between typical carcinoid tumor and small cell carcinoma have been called atypical carcinoids. However, these tumors were too heterogeneous to be grouped into a single category. Subsequently, Travis et al. [10] proposed a fourth category, large cell neuroendocrine carcinoma, which is characterized by a poorly differentiated and high-grade neuroendocrine tumor of the non–small cell type. Large cell neuroendocrine carcinomas have been categorized as being between atypical carcinoids and small cell carcinomas in terms of clinical aggressiveness [2, 5, 11].

The incidence of large cell neuroendocrine carcinoma in cases of resectable primary lung cancers is 2.8–3.1% [2, 3]. The clinical features of the 38 patients with large cell neuroendocrine carcinoma in our study—the mean age, predominance of men, and strong association with smoking—were similar to those previously documented in the literature [2, 10]. However, 31 patients (82%) were asymptomatic and had large cell neuroendocrine carcinoma detected incidentally, which is a larger percentage than the percentages previously reported [6, 7]. This difference is presumably due to the number of patients with earlier stages of large cell neuroendocrine carcinoma in our study population.

Large cell neuroendocrine carcinomas have been reported as occurring in either central or peripheral locations [10]. Our experience supports a predominately peripheral location. In our series, large cell neuroendocrine carcinomas were slightly more common

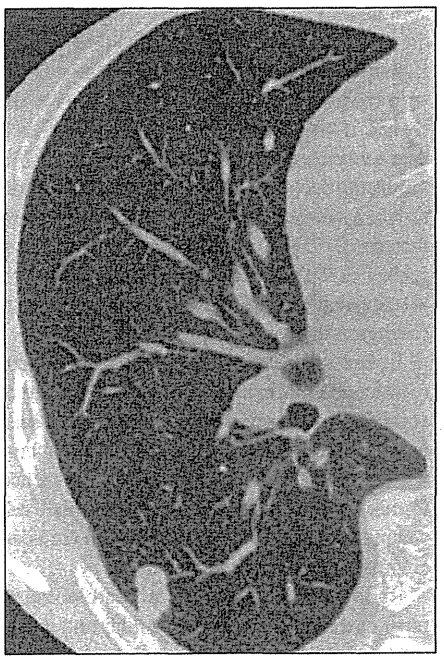

Fig. 1.—Thin-section (2-mm collimation) CT scan obtained with lung window setting in right lower lobe of 77-year-old man with large cell neuroendocrine carcinoma shows well-defined lobulated nodule.

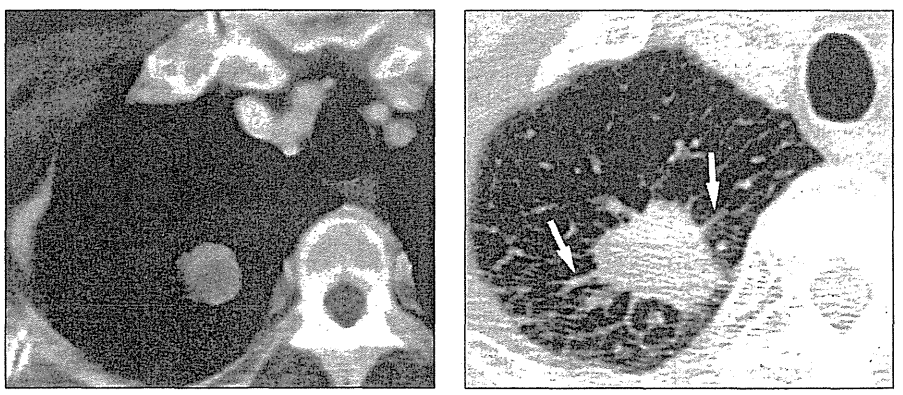

Fig. 2.—Large cell neuroendocrine carcinoma in 71-year-old-man.
A, Contrast-enhanced conventional 10-mm-thick CT scan obtained in right upper lobe shows round nodule with mild homogeneous enhancement.
B, Thin-section (2-mm collimation) CT scan obtained with lung window setting shows ill-defined spiculated nodule. Coarse spiculation (*arrows*) is evident around periphery of tumor with extension to pleural surface and into emphysematous lung.

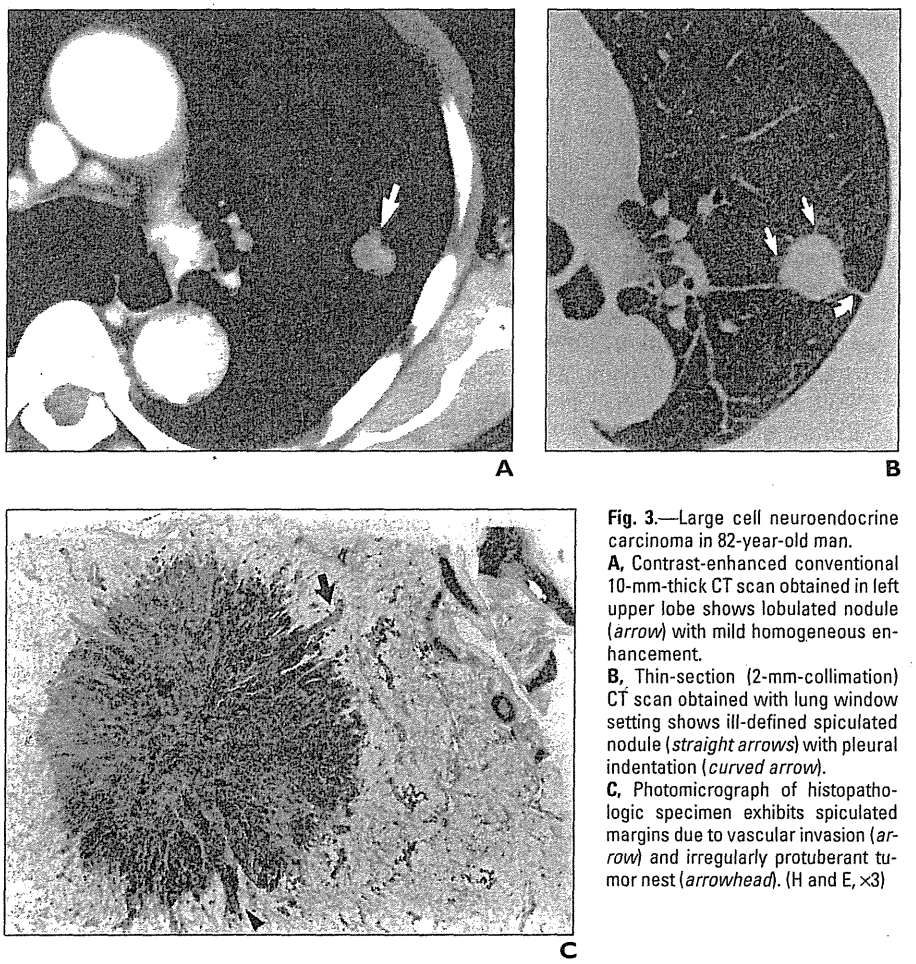

Fig. 3.—Large cell neuroendocrine carcinoma in 82-year-old man.
A, Contrast-enhanced conventional 10-mm-thick CT scan obtained in left upper lobe shows lobulated nodule (*arrow*) with mild homogeneous enhancement.
B, Thin-section (2-mm-collimation) CT scan obtained with lung window setting shows ill-defined spiculated nodule (*straight arrows*) with pleural indentation (*curved arrow*).
C, Photomicrograph of histopathologic specimen exhibits spiculated margins due to vascular invasion (*arrow*) and irregularly protuberant tumor nest (*arrowhead*). (H and E, ×3)

in the right lung than in the left and slightly more common in the upper lobe than in the middle or lower lobes. The reason for the higher incidence in these regions is uncertain; however, pulmonary carcinomas are known to occur with a relative frequency of 3:2 in the right versus left lung and in the upper versus lower lobe [12]. A well-defined and lobulated appearance without air bronchograms or calcification was the most common finding in the large cell neuroendocrine carcinomas observed in our study. These results are compatible with the recently reported descriptions of large cell neuroendocrine carcinoma [6, 7].

Jung et al. [7] reported that spiculation was present in eight (73%) of 11 cases of large cell neuroendocrine carcinoma. In our study, spiculation was found in six (32%) of 19 patients who had thin-section CT scans available. Spiculations are frequently seen in adenocarcinomas and occasionally in squamous cell carcinomas, corresponding to a diffuse desmoplastic response to tumor growth [13]. In our series, spiculated margins were caused by paracicatricial emphysema with extension into surrounding emphysematous lung or by linear opacities made more prominent by preexisting emphysema in four (67%) of the six tumors with spiculation. Because emphysema is thought to be caused by smoking, the spiculation of large cell neuroendocrine carcinomas in our study may be associated with the high incidence of smokers in our patients.

The scans of 13 of the 28 patients who had undergone contrast-enhanced CT showed inhomogeneous enhancement caused by gross intratumoral necrosis. Statistically, inhomogeneously enhanced tumors were larger than homogeneously enhanced tumors. Microscopic focal necrosis was present in each case, and the extent of necrosis varied considerably. In adenocarcinomas and squamous cell carcinomas, central necrosis is frequent and may be more extensive in larger tumors [12]. Similarly, in large-diameter large cell neuroendocrine carcinomas, the necrotic area seemed to be confluent and more extensive. Although small-diameter tumors contained macroscopic necrosis, they tended to show homogeneous enhancement. Identifying these necroses on contrast-enhanced CT was difficult because the necrotic foci were too small to be detected. Endobronchial growth has been associated with typical carcinoid tumors [12], but we observed it only infrequently in the large cell neuroendocrine carcinomas in our study. We found the incidence of postobstructive pneumonia or atelectasis on CT to be 8%, lower than the 27% (3/11 patients) reported by Jung et al. [7]. This difference in findings may be the result of infrequent central or endobronchial growth.

Before its new classification, large cell neuroendocrine carcinoma had been categorized in several ways. In reviewing previous cases seen in our institution, we noticed that some patients with large cell neuroendocrine carcinoma had formerly been diagnosed as having small cell carcinoma, adenocarcinoma, squamous cell carcinoma, or large cell carcinoma [3].

Radiographically, small cell carcinoma in most cases exhibits extensive hilar and mediastinal lymphadenopathy, and sometimes a primary pulmonary tumor is not recognized [12]. However, 5–10% of patients with small cell carcinoma have solitary pulmonary nodules [14–16]. Among eight patients in our study initially classified as having small cell carcinoma, six had been diagnosed as having

TABLE 2 Comparison of Macroscopic Necrosis and Tumor Size by Enhancement Pattern on Contrast-Enhanced CT in 28 Patients with Large Cell Neuroendrocrine Carcinoma

Feature	Enhancement Pattern		*p*
	Homogeneous	Inhomogeneous	
Macroscopic necrosis	7 of 15 patients	13 of 13 patients	< 0.003
Tumor size (mm)			
Mean ± SD	25 ± 10	51 ± 18	< 0.001
Range	13–54	20–92	

Note.—Overall mean tumor size ± SD was 32 ±19 mm.

an intermediate cell type of the disease. Yabuuchi et al. [16] reported that on CT, peripheral small cell carcinoma displays a well-defined, rounded, or lobulated homogeneous mass. The CT features on the scans of our patients initially diagnosed as small cell carcinoma were similar to the CT features of peripheral small cell carcinoma described earlier. Large cell neuroendocrine carcinoma and peripheral small cell carcinoma also have similar pathologic and CT features. Thus, histologic review of peripheral small cell carcinoma cases should be performed using new WHO criteria because it is possible that some of the patients with currently accepted diagnoses of peripheral small cell carcinoma should be reclassified as having large cell neuroendocrine carcinoma.

Our study has some limitations. The CT findings in our study population may not reflect the findings in all patients with large cell neuroendocrine carcinoma because our study was limited to patients whose tumors were surgically resected. This substantial selection bias might explain the presence of peripheral tumors in so many of our patients. However, a diagnosis of large cell neuroendocrine carcinoma is usually based on a histologic findings in a specimen obtained by surgical resection, as was the case in our study. More research is needed to clarify large cell neuroendocrine carcinoma findings in all patients, including patients with large cell neuroendocrine carcinoma who are not treated surgically. Other limitations of our study include a reliance on visual estimation of the CT scans with no comparison of the region of interest in the tumor with that in unenhanced scans and the variation in both the IV contrast injection rates (range, 1–2 mL/sec) and scanning times (range, 70–120 sec), each of which may have affected the homogeneity of the tumor.

In conclusion, we found that large cell neuroendocrine carcinoma usually appeared as a well-defined and lobulated tumor without air bronchograms or calcification. Infrequently, this appearance was accompanied by spiculation due to paracicatricial or preexisting emphysema. We found that the CT findings of large cell neuroendocrine carcinomas were similar to those of other expansively growing tumors, such as peripheral small cell carcinomas, poorly differentiated adenocarcinomas, and squamous cell carcinomas. Large-diameter large cell neuroendocrine carcinomas tended to show inhomogeneous enhancement because of necrosis, but this type of enhancement was not necessarily apparent in small-diameter (< 33 mm) large cell neuroendocrine carcinomas, even if the tumors were necrotic.

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