oncologists and surgeons. In general, we previously performed initial surgery for csN2 patients. However, from around the year 2000, csN2 patients have been treated by definitive CRT, similar to unresectable N2 disease, if pN2 status was confirmed by biopsy of the csN2 node. Although the CRT regimens were decided by the cancer board on a case-by-case basis, most fit patients received cisplatin and vinorelbine with concurrent thoracic radiotherapy (16). From around the year 2005, we chose initial surgery strategy for csN2 patients, because postoperative adjuvant chemotherapy became available (17).

Treatment and Histologic Evaluation

For patients who completed CRT, measurable lesions and objective tumor responses were defined according to Response Evaluation Criteria in Solid Tumors classification (18). Resected diseases were staged based on the sixth edition of the TNM classification of the Union for International Cancer Control (19). Histologic classification was determined according to the World Health Organization criteria (20).

Statistical Analysis

Student's *t*-test and Pearson's χ^2 test were used for comparison. The OS and recurrence-free survival (RFS) were calculated by applying the Kaplan–Meier method starting at the date of resection (initial surgery cases) or the first day of CRT (definitive CRT cases); the log-rank test was used to compare survival curves. The median follow-up period was 5.9 years (range, 1.8–12.6). Multivariate analysis was performed using the Cox proportional hazards model to identify independent prognostic factors. All *P* values were two sided, and a *P* value of <0.05 was considered to indicate a statistically significant difference. Statistical analyses were performed with the GraphPad Prism version 5.01 (GraphPad Software, San Diego, CA, USA) software and the JMP version 8.02 (SAS Institute, Inc., Cary, NC, USA) software.

RESULTS

Treatment Courses of the Patients with csN2 Disease

Figure 1 shows treatment courses of the 125 csN2 patients. A total of 97 patients (78%) underwent primary thoracotomy without preoperative histologic examination of csN2 nodes, and the remaining 28 patients received mediastinoscopic ($n = 26$) or thoracoscopic ($n = 2$) biopsy of csN2 nodes. In 5 of the 28 patients, N2 disease was histologically not identified, and they subsequently underwent thoracotomy. Among the remaining 23 patients whose pN2 status (csN2/pN2) was confirmed by biopsy, 18 patients underwent definitive CRT and 3 underwent induction chemotherapy followed by surgery.

Patient Characteristics and Final Pathologic N Status of csN2 Patients after Initial Surgery

Table 1 shows patient characteristics of csN2 patients who underwent initial surgery. Nine patients had unresectable disease (unresectable T4, $n = 3$; pleural dissemination, $n = 6$). Subsequently, 88 underwent lung resection. Pathologic examination revealed true (pathologic) N2 disease (csN2/pN2) in 45 (51%) patients. The remaining 43 (49%) patients had pN0 (csN2/pN0, $n = 31$) or pN1 (csN2/pN1, $n = 12$) disease. Among the 45 csN2/pN2 patients, true (pathologic) single-station N2 (csN2/psN2) was identified in 17 patients, and the remaining 28 patients had clinically undetected pathologic multistation N2 disease (csN2/pmN2). The positive predictive value of single-station N2 disease by contrast-enhanced CT was

Table 1. Patient characteristics of clinical single-station N2 (csN2) non-small-cell lung cancer (NSCLC) patients who underwent initial surgery

No. of patients	97
Age (year, median and range)	66, 29–84
Gender	
Male	74 (76)
Female	23 (24)
Histology	
Adenocarcinoma	52 (54)
Squamous-cell carcinoma	25 (26)
Adenosquamous carcinoma	7 (7)
Large-cell carcinoma	6 (6)
Others	7 (7)
Tumor location	
Upper lobe	63 (65)
Middle and lower lobes	34 (35)
Clinical T-factor	
T1	18 (19)
T2	56 (58)
T3	23 (23)
Mode of resection	
Puemonoectomy	14 (14)
Bi-lobectomy	8 (8)
Lobectomy	66 (69)
Bronchoplasty	5 (5)
Exploratory thoracotomy	9 (9)
Pathologic N-status[a]	
pN0	31 (35)
pN1	12 (14)
pN2	45 (51)
Pathologic single-station N2	17 (19)
Pathologic multistation N2	28 (32)

Numbers in parentheses are percentages.
[a]Percentages were calculated by using resected cases ($n = 88$).

19% (17/88). Mode of resections for 45 csN2/pN2 patients who underwent initial surgery included 33 (73%) lobectomies, 5 (11%) bi-lobectomies and 7 (16%) pneumonectomies. Two patients received bronchoplastic procedures too. One patient who underwent right lower lobectomy died in-hospital due to postoperative pneumonia. Adjuvant chemotherapy was given to recent three patients in this series: two received cisplatin and vinorelbine and one received carboplatin and paclitaxel.

Outcomes of csN2 Patients after Initial Surgery According to Final Pathologic N Status

The survival of csN2 patients after initial surgery according to final pathologic N status is shown in Fig. 2A and B. The patients whose mediastinal node involvement was not identified pathologically (csN2/pN0–1 patients) had a favorable prognosis compared with those whose N2 disease was pathologically confirmed (csN2/pN2 patients; 5-year RFS, csN2/pN0: 56.7%, csN2/pN1: 41.7%, csN2/pN2: 22.3%; 5-year OS, csN2/pN0: 58.1%, csN2/pN1: 50.0%, csN2/pN2: 23.6%). RFS and OS of the patients with csN2/pN2 were significantly and marginally worse than those of the patients with csN2/pN0 (RFS, $P < 0.001$; OS, $P < 0.001$) and csN2/pN1 (RFS, $P = 0.055$; OS, $P = 0.079$). For reference, 5-year RFS and OS rates of the csN2/pN2 patients who underwent definitive CRT in the same study period ($n = 18$) were 22.2 and 27.8%, respectively.

Recurrence Patters and Treatments for csN2/pN2 Patients after Initial Surgery

Recurrence developed in 32 (71%) patients. The patterns and treatments for initial recurrence are listed in Table 2. Locoregional and distant failures occurred almost equally. The treatment modalities for recurrence were CRT in 3 (9%), chemotherapy in 8 (25%) and local radiotherapy in 6 (19%), respectively. In this study, one patient received epidermal growth factor receptor tyrosine kinase inhibitor as an initial therapy for first recurrence.

Prognostic Factors in csN2/pN2 Patients after Initial Surgery

The results of univariate analysis by using the log-rank test are shown in Table 3. True (pathologic) single-station N2 status (csN2/psN2, $P = 0.024$), number of metastatic nodes

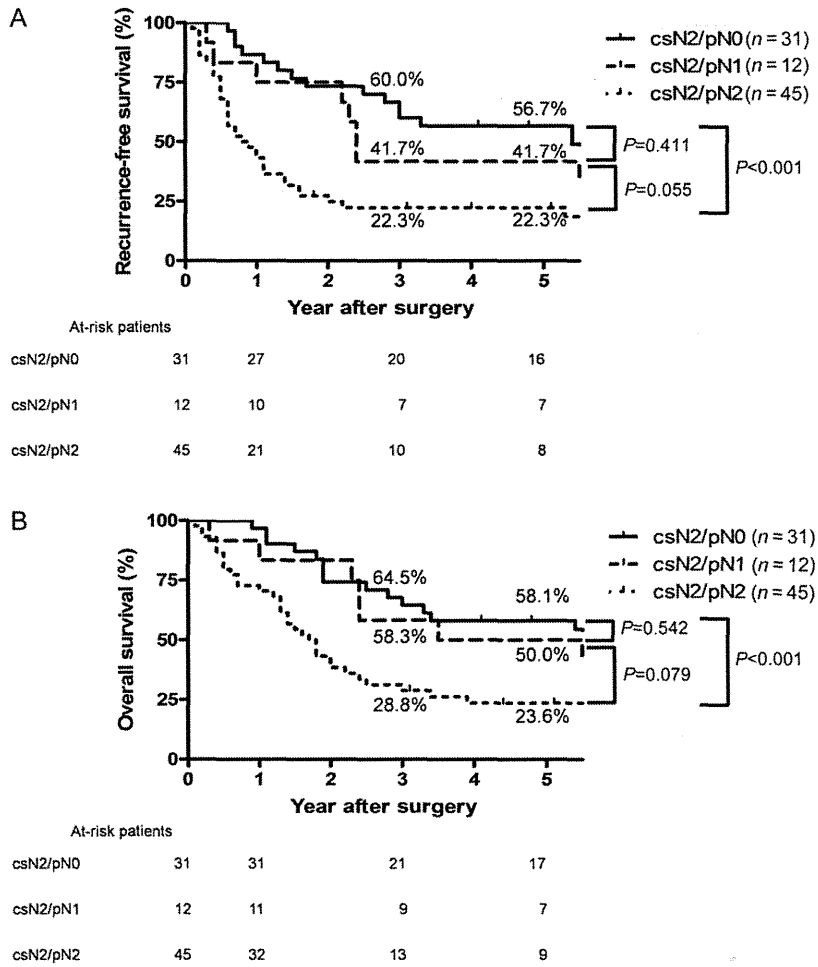

Figure 2. Recurrence-free (**A**) and overall (**B**) survival (RFS and OS) curves of clinical single-station N2 (csN2) patients who underwent initial surgery according to final pathologic N (pN) status.

Table 2. Recurrence patterns of csN2/pN2 NSCLC patients who underwent initial surgery

	csN2/pN2 patients ($n = 45$)
Recurrence	32 (71)
Locoregional	17 (38)
Lung	6 (14)
Mediastinal LN	8 (18)
Cervical LN	2 (4)
Pleural effusion	1 (2)
Distant	15 (33)
Brain	2 (4)
Bone	5 (11)
Abdominal LN	0
Multiple organs	8 (18)
Treatment of recurrence	
CRT	3 (9)
Chemotherapy	8 (25)
Radiotherapy[a]	6 (19)
Best supportive care	5 (47)

Numbers in parentheses are percentages.
LN, lymph node; CRT, chemoradiotherapy.
[a]Gamma knife therapy for brain metastasis is included.

Table 3. Univariate analysis of scN2/pN2 patients for identifying prognostic factors after initial surgery

Variable	*n*	5-year OS (%)	*P* value
Age (years)			
<70	29	21.4	0.433
≥70	16	25.0	
Gender			
Man	34	27.6	0.127
Woman	11	9.1	
Histology			
Adenocarcinoma	26	25.4	0.968
Others	19	19.7	
Mode of resection			
Pneumonectomy	7	28.6	0.762
Lobectomy	38	22.5	
Tumor location			
Upper lobe	29	29.2	0.208
Middle or lower lobe	16	12.5	
Tumor size (cm)			
≤3	7	68.6	0.106
>3	38	15.0	
Pathologic N2 status			
Single-station N2	17	38.1	0.024
Multistation N2	28	14.3	
Skip N2			
Yes	11	32.7	0.424
No	34	19.9	
Number of metastatic nodes[a]			
≤4	19	39.1	0.035
>4	24	12.5	
Metastasis in subcarinal station (#7)			
Yes	18	11.1	0.014
No	27	30.7	
Adjuvant chemotherapy			
Yes	3	NR	0.197
No	42	21.4	

OS, overall survival.
[a]Two patients were excluded from this analysis due to missing data.

(<4, $P = 0.035$) and a pathologic negative subcarinal (#7) station ($P = 0.014$) had a favorable relationship to OS. The 5-year OS rate of true (pathologic) single-station N2 disease (csN2/psN2, $n = 17$) was 38.1%. The N2 disease without concomitant N1 disease (skip N2) and small primary tumor size (≤3 cm) were not shown to be statistically significant. A multivariate analysis for OS identified that psN2 status (csN2/psN2) (hazard ratio [HR] = 0.35, $P = 0.008$) and pathologic negative #7 station (HR = 0.34, $P = 0.022$) were independent favorable prognostic factors of survival after initial surgery (Table 4). The survival curves of csN2/pN2 patients after initial surgery according to the status of these two prognostic factors are shown in Fig. 3A and B. Patients with the two factors (psN2/#7-negative, $n = 11$) revealed relatively favorable 5-year RFS and OS of 37.5 and 43.8%, respectively. In contrast, all patients without the two factors (pmN2/#7-positive, $n = 12$) developed recurrence within 1.1 years and died within 2 years of initial surgery. The survival curves of the patients with one of these factors crossed over each other, but the patients with psN2 limited in #7 station (psN2/#7-positive) tended to show a slightly favorable survival compared with pmN2 without #7 involvement (5-year RFS and OS, psN2/#7-positive: 33.3 and 33.3%, pmN2/#7-negative: 25.0 and 25.0%, respectively).

DISCUSSION

In the current study, of the 88 csN2 patients who underwent initial surgery, true (pathologic) N2 (csN2/pN2) status was identified in approximately half (51%) of the patients, while the remaining patients had pN0–1 (csN2/pN0–1) disease, with favorable prognoses. The positive predictive value of single-station N2 disease by contrast-enhanced CT was only 19% (17/88). CT scans still play a central role in lung cancer staging, but pooled data yielded a sensitivity of 57%, specificity of 82%, positive predictive value of 56% and negative predictive value of 83% (21). Yoshino et al. (22) recently analyzed the surgical outcomes of 800 NSCLC patients with

c-stage IIIA (cN2) disease diagnosed mainly by CT scans in the Japanese Lung Cancer Registry Study in 2004, and 346 (43.3%) patients actually had pN0–1 disease. These results imply that csN2 diagnoses by contrast-enhanced CT scans include considerable overdiagnoses and that we should not exclude csN2 patients from surgical candidate based only on CT findings.

Table 4. Prognostic factors of csN2/pN2 patients after initial surgery based on multivariate analyses for OS

Variable	HR	95% CI	*P* value
Number of metastatic nodes			
≤4 vs. >4	0.98	0.40–2.44	0.959
Pathologic N2 status			
Single-station N2 vs. multistation N2	0.35	0.15–0.76	0.008
Metastasis in subcarinal station (#7)			
No vs. yes	0.34	0.13–0.86	0.022

HR, hazard ratio; CI, confidence interval.

On the other hand, the survival of the csN2 patients whose N2 was pathologically confirmed after initial surgery (csN2/pN2 patients) was unsatisfactory. The 5-year RFS and OS were 22.3 and 23.6%, respectively. In the current study period, a total of 18 patients with the same N2 burden (csN2/pN2) underwent CRT, a standard treatment modality especially in western countries. Their 5-year RFS and OS were 22.2 and 27.8%, respectively. This was a retrospective small study and we could not compare the survival results for csN2/pN2 patients after initial surgery and CRT. However, the current results imply that the western guideline recommending CRT for cN2/pN2 NSCLC also applies to csN2/pN2 disease. The

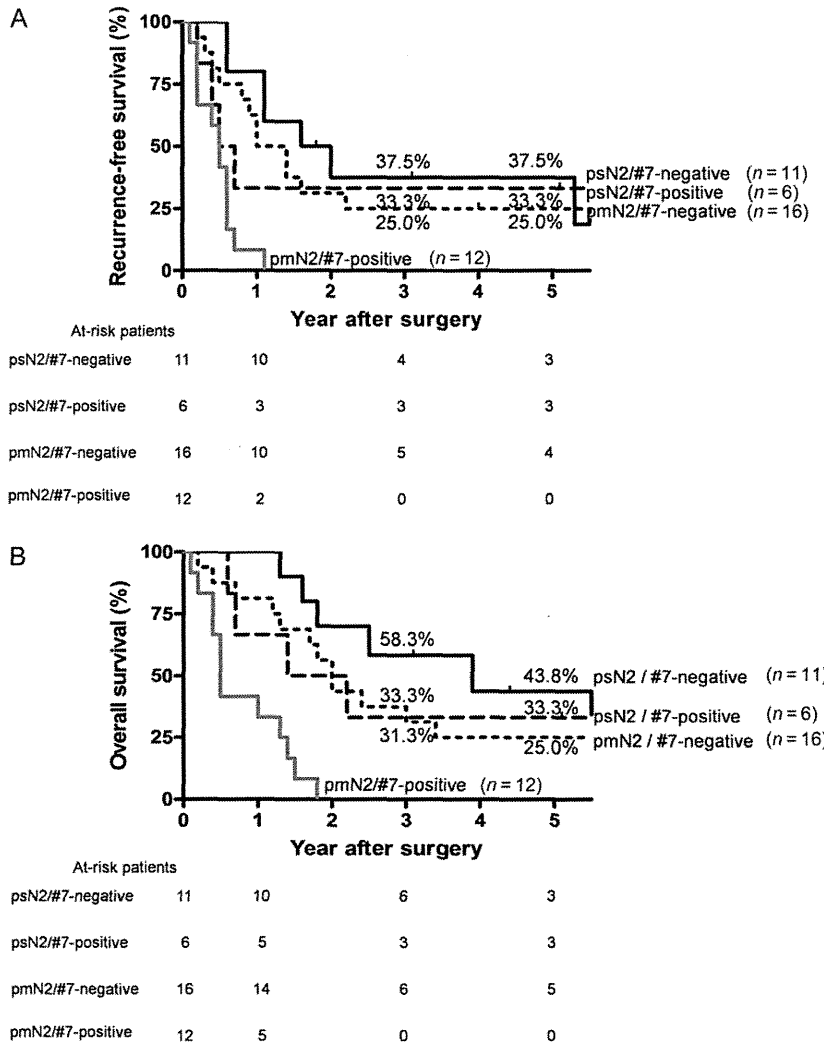

Figure 3. RFS (**A**) and OS (**B**) curves of csN2/pN2 patients after initial surgery based on the status of two prognostic factors. The patients with pathologic single-station N2 (psN2) and negative subcarinal station (#7) ($n = 11$) revealed a relatively favorable 5-year OS of 43.8%. The patient with both pathologic multistation N2 (pmN2) and positive #7 node ($n = 12$) revealed an extremely poor survival and all died within two postoperative years. The patients with either factor (psN2/#7-positive or pmN2/#7-negative) revealed also poor survival.

postoperative survival rates of csN2/pN2 patients varied depending on the two favorable prognostic factors identified by multivariate analysis: psN2 status and negative subcarinal (#7) station. The patients having csN2/psN2 without subcarinal involvement (#7-negative csN2/psN2) revealed a favorable 5-year OS of 43.8% after initial surgery. Although the survival data of CRT for this subgroup are not available, these results suggest that initial surgery is a treatment of choice if the patients with true single-station N2 status and negative subcarinal involvement are preoperatively confirmed.

The two prognostic factors for resected csN2/pN2 NSCLC patients, psN2 status and negative subcarinal station, have been reported as favorable prognostic factors for resected 'cN0–1/pN2' NSCLC patients in many studies (9–14,23,24). In the Japanese Lung Cancer Registry Study in 2004, psN2 status was also shown to be a significant favorable prognostic indicator in resected 436 'cN2/pN2' NSCLC patients (5-year OS, cN2/psN2 35.8%, cN2/pmN2 22.0%, $P = 0.0053$) (22). Regnard et al. (23) analyzed the survival of 254 pN2 NSCLC patients and demonstrated that pN2 patients with negative subcarinal node had a 5-year OS of 34%. In the current study, both two factors were also important for 'csN2/pN2' patients after surgery. N2 disease without concomitant N1 involvement (skip N2) and smaller size of primary tumor (lower T-classification) have also been reported to be prognostic factors (12,24), but these were not shown to be statistically significant in the current study, possibly due to the small sample size.

Initial surgery may be beneficial to csN2/psN2 without subcarinal involvement, but such patients accounted for only in 13% (11/88) of the resected csN2 NSCLC patients in our cohort. Clinically undetectable pmN2 disease (csN2/pmN2) was observed in 32% (28/88). On the other hand, it is also notable that 49% (43/88) of the resected csN2 diseases were pN0–1 diseases. Additional diagnostic modalities including PET combined with CT (PET–CT), mediastinoscopic biopsy or endobronchial ultrasound-guided (EBUS) biopsy are mandatory for csN2 diseases diagnosed by CT, in order to reduce overdiagnoses and select appropriate surgical csN2/pN2 candidates, #7-negative csN2/psN2 disease. PET–CT has been widely used as a non-invasive staging modality of NSCLC (21,25). Due to its high negative predictive value of over 90% (25), when csN2 node detected on CT is negative on PET–CT, which suggests pN0–1, initial surgical resection may be the treatment of choice. Also, when PET–CT reveals negative #7 station in csN2 patients, initial surgical resection may be justified. In the current series, six patients were diagnosed as csN2 not only by CT but also by PET–CT. Among them, five actually had psN2. In contrast, the positive predictive value of PET–CT scan is only ~70% (25). If other positive mediastinal nodes were depicted for csN2 patients on PET–CT, the exact N2 status (single or multistation) should be confirmed by biopsy. csN2 patients whose N2 status proved to multistation by biopsy should be indicated for definitive CRT or induction therapy followed by surgery. Although mediastinoscopic biopsy remains to be the gold standard of invasive staging, recent minimally invasive endoscopic techniques including EBUS transbronchial needle aspiration have been proved to replace mediastinoscopic biopsy because of the similar high performance reported in determining the true pathologic N status (26) and should be used more in clinical practice.

This study includes some limitations. First, this is a retrospective study based on a small cohort in a single institution. Secondly, csN2 patients were not randomly allocated to between initial surgery and other treatment modalities. The study population included selection bias, and we could not evaluate the significance of initial surgery for csN2 patients relative to CRT, a standard treatment modality especially in western countries. Due to these limitations, it may be inadequate to draw definite conclusions from our results. However, our results clearly indicated that true csN2 patients without subcarinal involvement among csN2 patients could achieve relatively long-term survival (5-year OS: 43.8%) by initial surgery. Nowadays, cisplatin-based adjuvant chemotherapy has been a standard of care for resected pN2 NSCLC patients. Further study should be conducted focusing on whether adjuvant chemotherapy produces further survival improvement for this csN2 population (#7-negative true csN2).

In conclusion, as this retrospective study showed that approximately one half of the csN2 NSCLC patients actually had pN0–1 diseases, we should not exclude surgical resection as their treatment option based only on CT findings. However, the csN2/pN2 patients revealed heterogeneous outcomes after initial surgery in terms of their prognosis. Initial surgery is indicated for csN2/pN2 patients if the true single-station csN2/pN2 without subcarinal involvement is preoperatively confirmed.

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Conflict of interest statement

None declared.

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Original contribution

Distinct clinicopathologic characteristics of lung mucinous adenocarcinoma with *KRAS* mutation ☆

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Summary Primary mucinous adenocarcinomas are uncommon, and their pathogenesis remains unclear. We recently reported the clinicopathologic characteristics of surgically resected mucinous adenocarcinoma, including the frequent involvement of the left and lower lung and absence of central fibrosis. The present study attempted to clarify the pathogenesis of mucinous adenocarcinoma based on *KRAS* mutation status. We selected 45 mucinous adenocarcinoma cases from among 2474 surgically resected primary lung adenocarcinomas. Of these, 22 had a *KRAS* mutation (48.9%), whereas only 7 (15.6%) had an *epidermal growth factor receptor* mutation, and 2 cases had both mutations. The mucinous adenocarcinomas with *KRAS* mutations were located in the lower lung lobe significantly more often ($P < .05$) than were tumors without *KRAS* mutation. The mucinous adenocarcinoma cases with *KRAS* mutations also had a significantly lower frequency of nuclear atypia ($P < .05$). We compared the degree of immunostaining for matrix metalloproteinase-7 (MMP-7), laminin-5, and geminin in the mucinous adenocarcinoma with and without *KRAS* mutation. The proportion of geminin-positive cells was lower among the cases with a mutation than among those without (0.7% versus 2.1%; $P < .05$). No significant differences in the extent of staining of the other markers were observed between the groups. The current study clearly demonstrated that mucinous adenocarcinomas with *KRAS* mutations have clinicopathologic characteristics different from those of mucinous adenocarcinoma without such mutations.

1. Introduction

Lung adenocarcinomas are characterized by a high degree of morphologic heterogeneity. With reference to the cytologic features of the cells, Shimosato et al [1] and Kimula [2] subclassified primary tumor cells into 5 subgroups: goblet, bronchial cell surface type, bronchial gland, Clara, and type II

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pneumocyte. In recent years, lung adenocarcinoma composed predominantly of goblet cells has been classified as mucinous adenocarcinoma (MA) [3]. Previous authors have reported that MA is less frequently associated with lymph node metastasis than other adenocarcinomas but is associated with lobar pneumonic clinical features [4]. Wislez et al [5] reported that MA appears to be resistant to epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitors (TKIs). On the other hand, they may be more sensitive to taxane [6,7].

Malignant neoplasms are considered to develop through the accumulation of genetic abnormalities. In lung cancer, *KRAS* and *EGFR* mutations frequently are detected. *KRAS* mutation is the most common driver mutation in human cancers, although the prevalence of mutations and the affected codons differ according to the type of cancer. In lung adenocarcinoma, the frequency of *KRAS* mutation–positive cases is 5% to 40%, and mutations are more common in male patients and in smokers [8-12]. On the other hand, *EGFR* mutation has been an area of particular interest because of the finding that the administration of EGFR-TKIs in clinical trials resulted in a high response rate among patients with lung adenocarcinoma carrying an *EGFR* mutation. These mutations were correlated with the observed clinical characteristics of responders to TKIs, including female sex, Asian ethnicity, an absent or infrequent smoking history, and a diagnosis of lung adenocarcinoma [13-15]. Furthermore, mutations in *KRAS* and *EGFR* usually are mutually exclusive.

We recently reported that the clinicopathologic characteristics of MA include the frequent involvement of the left and lower lung and absence of central fibrosis [4]; these adenocarcinomas form a distinct subset and should be considered different from other lung adenocarcinomas. The present study attempted to clarify the pathogenesis of MA with special reference to *KRAS* and *EGFR* mutation status. We analyzed the correlations between the mutations and clinicopathologic characteristics.

2. Materials and methods

2.1. Patients

Between August 1992 and March 2010, a total of 2474 patients with primary adenocarcinoma of the lung were treated surgically in the Division of Surgery of the Department of Thoracic Oncology, National Cancer Center Hospital East, Chiba, Japan. From these, 45 MAs (2.1%) were selected for study. The data collection and analyses were approved by the institutional review board, and the need to obtain informed consent from each patient was waived.

2.2. Clinical information

All available clinical information was obtained from the clinical records and reports completed by the referring physicians. The records were reviewed for patient age; sex; smoking index; and tumor size, stage, and location.

2.3. Pathologic information

The resected tissues had been fixed in 10% formalin or absolute methyl alcohol and embedded in paraffin. Serial 4-μm sections were stained with hematoxylin and eosin using the Alcian blue–periodic acid Schiff method to visualize cytoplasmic mucin production or the Victoria van Gieson method to identify elastic fibers. The tumors were classified according to the criteria of the current histologic classification adopted by the World Health Organization [16]. Tumor cells of MA have a goblet or columnar morphology with abundant intracytoplasmic mucin. The apex of each cell typically is occupied by a large mass of mucin, and the carcinoma cells show mucin production with mucus pooling in the surrounding alveolar spaces. [3,17-20].

The 45 MAs examined in this study were classified into 3 groups: mucinous adenocarcinoma in situ (size $\leq$3 cm; n = 24), mucinous minimally invasive adenocarcinoma (size $\leq$3 cm; n = 1), and invasive mucinous adenocarcinoma (size >3 cm; n = 20). Nuclear atypia was said to be present when there was nuclear enlargement, prominent nucleoli, or overlapping nuclei (Fig. 1). Pathologic staging was performed according to the classification of the Union for International Cancer Control [21].

2.4. Antibodies and immunohistochemical staining

Immunohistochemical staining was performed according to the method previously reported [22]. We used 3 antibodies: laminin-5 subunit γ2 (clone D4B5; Chemicon, Temecula, CA), MMP-7 (matrilysin, clone 141-7B2; Daiichi Fine Chemical, Toyama, Japan), and geminin (clone EM6; Novocastra, Newcastle-upon-Tyne, UK). We used the immunohistochemical status of laminin-5, MMP-7, and geminin to evaluate the proliferative ability and invasive capacity of the cancer cells. Basement membranes of bronchial epithelium, lymphocytes in germinal centers, and human lung adenocarcinomas were used as positive controls for laminin-5, geminin, and MMP-7, respectively. As the negative control, we added diluent containing 10% swine serum and confirmed that no nonspecific reaction was shown.

2.5. Staining scores

All the stained tissue sections were scored semiquantitatively and evaluated independently under a light microscope by 2 observers (H. I. and G. I.) with no knowledge of the clinicopathologic data. When the evaluations differed, the tissues were examined by both observers through a multiheaded conference microscope, and a consensus was reached. The labeling scores, except for geminin, were calculated by multiplying the percentage of stained tumor cells per lesion (0%-100%) by the staining intensity (0,

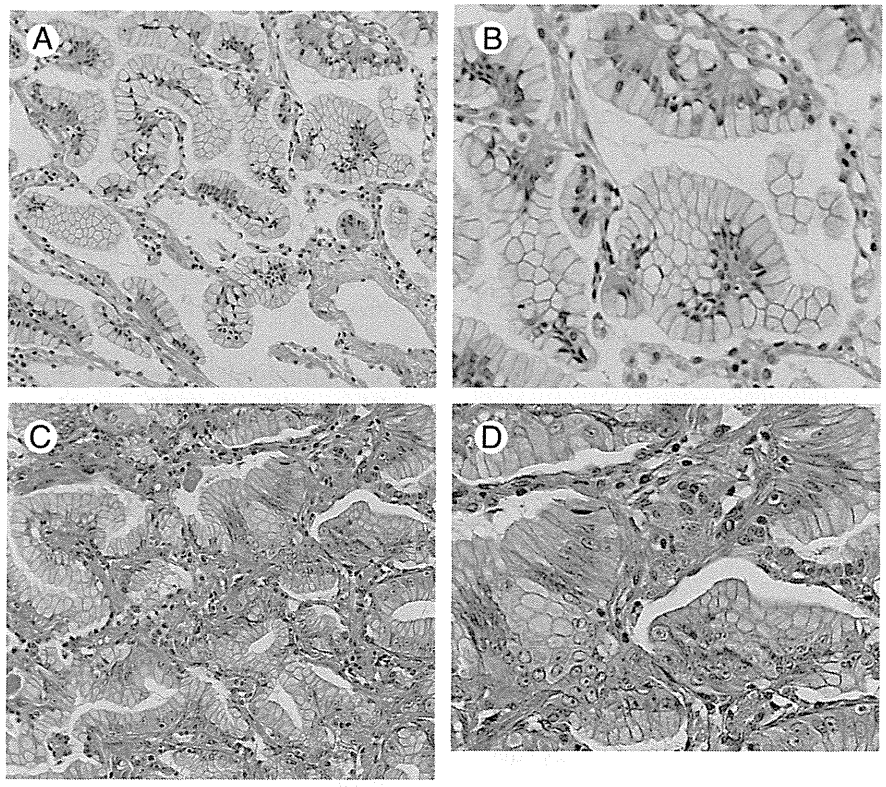

Fig. 1 Typical morphology of MA, characterized by tall columnar cells with basal nuclei and pale cytoplasm and various amounts of cytoplasmic mucin. Apex of each cell is occupied by large mass of mucus, which compresses nucleus toward basal end of the cell. A and B, Tumors with *KRAS* mutation: nuclear enlargement, prominent nucleoli, and overlapping nuclei are rarely observed (A, ×10; B, ×40). C and D, Tumors without *KRAS* mutation: nuclear enlargement, prominent nucleoli, and overlapping nuclei are visible (C, ×10; D, ×40).

negative; 1, weak; 2, strong), with the resulting possible scores ranging from 0 to 200. In the case of geminin, the number of positively stained cells per 1000 tumor cells was counted. Two independent observers' average was assigned as the staining score for each case. The weighted κ statistics for laminin-5, MMP-7, and geminin were 0.701, 0.734, and 0.842, respectively.

2.6. *KRAS* and *EGFR* mutation analysis

We isolated DNA from formalin-fixed, paraffin-embedded surgical specimens. The tumor samples were mapped using hematoxylin and eosin staining and manually macrodissected with a needle, and genomic DNA was isolated using the QIAamp DNA FFPE Tissue Kit (Qiagen, Venlo, The Netherlands). The *KRAS* mutation in codons 12 and 13 of the exon 2 fragment was amplified according to a previously described method [9-11,23,24]. The *EGFR* exons 18 to 21 were amplified largely according to a previously described method [10,24-26].

2.7. Statistical analysis

The Fisher exact probability test was used to compare binomial proportions. The Mann-Whitney *U* test was used to compare the staining scores. $P < .05$ was considered statistically significant. All the statistical analyses were performed using SPSS version 11.0 (SPSS, Inc, Chicago, IL).

3. Results

3.1. Clinicopathologic findings

The clinicopathologic data for the MA cases are summarized in Table 1. Of the 45 patients, 19 were men and 26 were women, and 17 of the patients (38%) were smokers (smoking index ≥200; 13 men, 4 women). The maximum diameters of the tumors ranged from 0.7 to 19.5 cm. Pleural invasion was observed in 1 case (2%). Lymphatic invasion likewise was present in 1 case. Pulmonary metastasis and vascular invasion were not observed.

3.2. *KRAS* and *EGFR* mutational analysis

The *KRAS* and *EGFR* mutational status of the 45 MA cases is shown in Table 2. *KRAS* mutation was identified in 22 cases (49%). The most frequent mutation was G12V (14 patients), followed by G12C (5 patients), and G12D (3

Table 1 Clinicopathologic data of 45 mucinous adenocarcinomas

Parameter	n (%)
Age (y)	
<65	19 (42)
≥65	26 (58)
Sex	
Male	19 (42)
Female	26 (58)
Smoking index	
<200	28 (62)
≥200	17 (38)
Tumor size (cm)	
≤3	30 (67)
>3	15 (33)
Location	
Right	20 (44)
Left	25 (56)
Lobe	
Upper/middle	10 (22)
Lower	35 (78)
Pathologic T stage	
1	30 (67)
2-4	15 (33)
Pathologic N stage	
0	45 (100)
1-3	0
Pathologic stage	
1	39 (87)
2-4	6 (13)
Pleural invasion	1 (2)
Pulmonary metastasis	0
Lymphatic permeation	1 (2)
Vascular invasion	0
Nuclear atypia	20 (44)
Central fibrosis	7 (16)

patients). No mutation in codon 13 was found. Mutations of *EGFR* were identified in 7 cases (16%). All were detected in exon 21 (L858R). That is, mutations in exons 19, 20, and 22 were not found. Two patients had concomitant *KRAS* and *EGFR* mutations. The specific mutations were a *KRAS* mutation at codon 12 (G12V) and an *EGFR* mutation within exon 21 (L858R). A case with pleural invasion exhibited *KRAS* mutation G12C. A case with lymphatic permeation did not exhibit either *KRAS* or *EGFR* mutation.

Table 2 *KRAS* and *EGFR* status of 45 mucinous adenocarcinomas

Gene	Mutation	n (%)
KRAS	G12V	14 (64)
	G12C	5 (23)
	G12D	3 (13)
	None	23
EGFR	L858R	7 (100)
	None	38

3.3. Clinicopathologic comparison of MA cases with or without *KRAS* mutation

The MA cases with *KRAS* mutation were significantly more likely to be located in the lower pulmonary lobe ($P < .05$) than were tumors without the mutation (Table 3). The tumors with *KRAS* mutation (Fig. 1A and B) showed significantly less nuclear atypia (enlargement, prominent nucleoli, and overlapping nuclei) ($P < .05$) than cases without *KRAS* mutation (Fig. 1C and D).

3.4. Immunohistochemical comparison of MA cases with or without *KRAS* mutation

Because nuclear atypia is a feature of malignancy, we assumed that the proliferation ability and the invasive capacity of MA cases with and without *KRAS* mutation

Table 3 Relation between *KRAS* mutation and mucinous adenocarcinoma features

	KRAS mutation (%)		*P*
	Negative (n = 23)	Positive (n = 22)	
Age (y)			.18
<65	12 (52)	7 (32)	
≥65	11 (48)	15 (68)	
Sex			.87
Male	10 (43)	9 (41)	
Female	13 (57)	13 (59)	
Smoking index			.68
<200	15 (65)	13 (59)	
≥200	8 (35)	9 (41)	
Tumor size (cm)			.84
≤3	15 (65)	15 (68)	
>3	8 (35)	7 (32)	
Location			.297
Right	12 (52)	8 (36)	
Left	11 (48)	14 (64)	
Lobe			.0083
Upper/middle	8 (35)	2 (9)	
Lower	15 (65)	20 (91)	
Pathologic T stage			.84
1	15 (65)	15 (68)	
2-4	8 (35)	7 (32)	
Pathologic N stage			1
0	23 (100)	22 (100)	
1-3	0	0	
Pathologic stage			.67
1	19 (83)	20 (91)	
2-4	4 (17)	2 (9)	
Pleural invasion	0	1 (5)	.31
Pulmonary metastasis	0	0	1
Lymphatic permeation	1 (4)	0	.33
Vascular invasion	0 (0)	0 (0)	1
Nuclear atypia	15 (65)	5 (23)	.0034
Central fibrosis	5 (22)	2 (9)	.25

Table 4 Relation between *KRAS* mutation and marker expression

	KRAS mutation		*P*
	Negative (n = 23)	Positive (n = 22)	
Laminin			.27
Mean	7.6	5.5	
Range	0-50	0-50	
MMP-7			.35
Mean	27	20	
Range	0-80	0-50	
Geminin			.003
Mean	2.1	0.69	
Range	0.25-7.42	0.16-2.44	

were different. Laminin-5 and MMP-7 expressions are reported to be associated with invasive capacity of tumor cells, whereas geminin expression is closely associated with proliferation (Table 4). The mean staining scores for laminin-5 in the MA cases with and without *KRAS* mutation were 5.5 and 7.6, respectively. The mean staining scores for MMP-7 cases and those without *KRAS* mutation were 19.5 and 27.0, respectively. There were no significant differences in the expression of laminin-5 or MMP-7 in the 2 groups. The mean positive rates for geminin in the MA cases with and without *KRAS* mutation were 0.7 and 2.1, respectively. The positive rate for geminin in the MA cases with *KRAS* mutation was significantly lower than that in cases without *KRAS* mutation ($P < .05$; Fig. 2).

4. Discussion

This is the first report of the relation between *KRAS* mutation status and the clinicopathologic features of MA. The tumors with *KRAS* mutation were significantly more likely to be located in the lower lobe ($P < .05$) than tumors without *KRAS* mutation. The MA cases with *KRAS* mutations also showed a significantly lower frequency of nuclear atypia ($P < .05$) than cases without. Moreover, the proportion of cells in the S, G_2, and M phases (geminin-positive cells) among the cases with *KRAS* mutation was significantly lower than among the cases without a mutation ($P < .05$). Therefore, the pathogenesis of MA may be classifiable into 2 groups according to *KRAS* mutation status.

In general, activated *KRAS* enhances cell proliferation. In lung tumors, *KRAS* mutations are thought to occur early in the genesis of adenocarcinomas, as they are found in 25% to 35% of atypical adenomatous hyperplasia, the precursor of certain types of adenocarcinomas [27,28]. On the other hand, Okudela et al [29,30] demonstrated that the forced expression of oncogenic *KRAS* induced severe growth suppression in immortalized human airway epithelial cells. Although the above findings were based on in vitro observations, these results might explain our current report in that the positive rate for geminin in MA cases with *KRAS* mutation was significantly lower than that in cases without *KRAS* mutation.

Type 1 congenital cystic adenomatoid malformations (CCAMs) are composed of one or more cysts greater than 2 cm in diameter surrounded by often underdeveloped alveolar parenchyma and a variable number of smaller cysts. In approximately one-third of cases, papillary tufts of cytologically bland mucinous cells punctuate the cyst surface [31,32]. Lantuejoul et al [33] suggested that mucinous cells in type 1 CCAM may represent mucinous bronchioloalveolar carcinoma (BAC) precursors. In mucinous cell clusters associated with CCAM, *KRAS* mutations

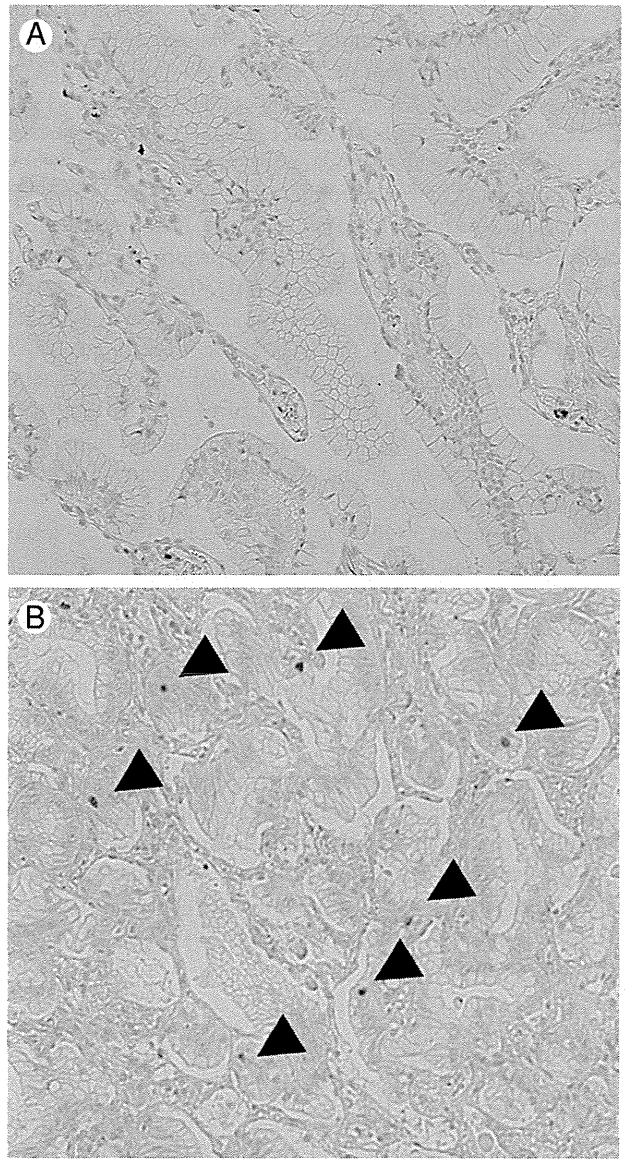

Fig. 2 Immunostaining for geminin in MA with different *KRAS* mutation status (×10). A, Tumor with *KRAS* mutation: geminin-positive rate is 0. B, Tumor without *KRAS* mutation: scattered geminin-positive cells (arrowheads) are visible (positive rate, 9.0).

were detected in 5 of 6 cases, whereas no *EGFR* mutations were reported. Furthermore, all mucinous cell cluster lesions displayed a staining pattern similar to that of mucinous BAC. These findings suggested that type 1 CCAM mucinous cells represent mucinous BAC precursors. Actually, CCAM also exhibits greater likelihood of being located in the lower lobe, similar to MA with a *KRAS* mutation in the current study. Among our 45 cases, however, no findings similar to those of CCAM were seen in the noncancerous fields. Further examination is needed to determine the relation between the pathogenesis of CCAM and of MA with a *KRAS* mutation.

Mutations of *KRAS* and *EGFR* in lung adenocarcinoma are reportedly found in a mutually exclusive manner [15,24,34]. However, a few cases with double mutations have been described. Among the 45 lesions in the present series, 2 had concomitant *EGFR* and *KRAS* mutations. These 2 cases displayed a *KRAS* mutation at codon 12 (G12V) and an *EGFR* mutation within exon 21 (L858R). The 2 cases did not have any clinical features in common.

Mutations of *EGFR* were identified in 7 (16%) of the 45 MA cases. No distant recurrences were observed in the 7 cases with these mutations. Of the 7 patients, 2 were men and 5 were women (male/female ratio, 1:2.5). Two of the patients (29%), both men, were smokers (smoking index $\geq$200). Compared with the MA cases without an *EGFR* mutation, the proportions of females and nonsmoker status were higher. Although the number of cases was limited, it appears that MA with *EGFR* mutation exhibits clinicopathologic characteristics similar to those of general primary lung adenocarcinomas.

In the current study, lower lobe location, a lesser frequency of nuclear atypia, and a smaller proportion of geminin-positive cells were characteristic features of MAs with *KRAS* mutations. However, they might be general characteristics of lung adenocarcinoma with this mutation. To rule out this possibility, it would be important to examine the clinicopathology of non-MA with *KRAS* mutation in the resectable cases.

In conclusion, we found that MAs with *KRAS* mutations exhibit different clinicopathologic characteristics than MAs without such mutations. Although further studies involving a larger number of cases are warranted, we believe that MA of the lung could be subclassified into distinct subtypes with different modes of pathogenesis according to *KRAS* mutation status.

Acknowledgments

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Original Study

The Differences of Biological Behavior Based on the Clinicopathological Data Between Resectable Large-Cell Neuroendocrine Carcinoma and Small-Cell Lung Carcinoma

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Abstract

Few reports elucidated the biological differences between resectable large-cell neuroendocrine carcinoma (LCNEC) and small-cell lung carcinoma (SCLC). We reviewed the clinical data of 140 patients with resected high-grade neuroendocrine carcinomas (NECs) and analyzed the clinicopathological features in relation to their survival. We demonstrated there were no apparent differences in biological behavior between pure and combined subtypes in high-grade NEC, and there were significant differences in prognostic factors between LCNEC and SCLC.

Introduction: Large cell neuroendocrine carcinoma of the lung and SCLC are collectively classified as high-grade NECs. However, there have been few reports focusing on the differences of clinicopathological prognostic factors between resectable LCNEC and SCLC. **Patients and Methods:** We reviewed the clinical data of 140 patients who underwent complete resection of high grade NEC in our institute and analyzed the clinicopathological features in relation to their survival. **Results:** There were no statistically significant differences in overall and recurrence-free survival between pure and combined subtypes in either LCNEC or SCLC. In LCNEC, larger tumor diameter ($P = .01$), nodal metastasis ($P < .01$), lymphatic permeation ($P < .01$), and vascular invasion ($P = .01$) were unfavorable prognostic factors. However, in SCLC, tumor diameter and vascular invasion were not prognostic factors, but nodal metastasis ($P < .01$) and lymphatic permeation ($P = .03$) were strongly correlated with poor prognosis. **Conclusion:** There were no apparent differences in biological behavior between pure and combined subtypes in either LCNEC or SCLC. Lymphatic involvement was an important unfavorable prognostic factor in SCLC, whereas tumor diameter, vascular invasion, and lymphatic involvement had a poor prognostic effect in LCNEC.

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Keywords: Biological behavior, High grade neuroendocrine carcinomas, Large-cell neuroendocrine carcinoma, Pathological diversity, Prognostic factors, Small-cell lung carcinoma

Introduction

Large-cell neuroendocrine carcinoma (LCNEC) of the lung and small-cell lung carcinoma (SCLC) are collectively classified as high-grade neuroendocrine carcinomas (NECs) of the lung.[1-5] Since the histological entity of LCNEC was introduced in the World Health Organization (WHO) classification of 1999, the clinicopathological characteristics of LCNEC have been clarified, particularly with regard to patients' prognoses.[6-12] In contrast, SCLC is a common pulmonary neuroendocrine tumor, and patients with SCLC generally have very poor prognoses. Few reports have been published on the biological characteristics of resected SCLC, such as unfavorable prognostic factors, because surgical resection is indicated only for clinical stage I SCLC. Although the pathological diagnostic criteria of LCNEC have been established, it can be very difficult to distinguish between LCNEC and SCLC in some NECs. LCNEC shares many similarities with SCLC in histological, biological, molecular biological, and clinical aspects. The

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immunohistochemical and genetic similarities and differences between LCNEC and SCLC have been reported,[13] but the histopathological similarities and overlap in clinical characteristics have raised doubts over the distinction between LCNEC and SCLC, and led to proposals that the 2 types should be reclassified as a single group of high-grade NECs.[14,15]

High-grade NEC often includes other histological subtypes. When LCNEC has components of other non–small-cell lung cancer (NSCLC) types, it is classified as combined LCNEC. If LCNEC or other NSCLC components are included in SCLC, it is classified as combined SCLC. However the biological difference between pure and combined high-grade NECs have yet to be elucidated.

The aims of this study were to examine the biological differences between pure and combined high-grade NEC and to compare the difference of the clinicopathological prognostic features of resectable LCNEC and SCLC.

Patients and Methods

A total of 140 patients who underwent complete resection of high-grade NEC from January 1995 through December 2010 at the National Cancer Center Hospital East, Kashiwa, Japan, were enrolled in this retrospective study. All the patients had a solitary lesion, and patients who had received preoperative chemotherapy or thoracic radiotherapy were excluded. The preoperative evaluation included physical examination, blood chemistry analysis, bronchofiberscopy, chest radiography, computed tomography (CT) examinations of the chest, magnetic resonance imaging of the brain, bone scintigraphy, and positron emission tomography (PET), or combined PET-CT. If we needed to evaluate nodal metastasis, mediastinoscopy was performed. All patients underwent lobectomy or pneumonectomy and lymph node dissection. After surgery, SCLC patients were given adjuvant chemotherapy consisting of 4 cycles of cisplatin or carboplatin and etoposide when possible. For LCNEC patients, however, adjuvant chemotherapy was planned as in NSCLC patients. We surveyed the patients at 3-month intervals for the first 2 years and at 6-month intervals thereafter. The follow-up evaluation included physical examination, chest CT, and blood examination. Whenever any symptoms or signs of recurrence were detected, further evaluations were performed. We diagnosed recurrence on the basis of diagnostic imaging findings, and confirmed the diagnosis histologically when clinically feasible.

Data collection and analyses were approved and, because the research was a retrospective chart and specimen review and no personally identifiable information was included, the need to obtain written informed consent from each patient was waived by the institutional review board in March 2012.

We reviewed all the available pathology slides of resected specimens in this study. After fixing the specimens with 10% formalin and embedding them in paraffin, serial 4-μm sections were stained with hematoxylin and eosin. The sections were reviewed by 3 observers, who were blinded to patient identity. In some cases that were difficult to diagnose definitely, we consulted the other expert pathologists and a consensus diagnosis was reached. Formalin-fixed paraffin sections were stained for a panel of neuroendocrine markers, including a polyclonal antichromogranin A antibody (Ventana Medical Systems), CD56 (neural adhesion molecule) antibody (Nippon Kayaku), and monoclonal antisynaptophysin antibody (Dako), to confirm neuroendocrine features. Immunohistochemically, neuroendocrine differentiation was considered positive if the tumor cells exhibited focal, patchy, or diffuse staining in the intracellular areas for 1 or more of these 3 antibodies. We excluded large cell carcinomas with a neuroendocrine phenotype, which were negative on immunohistochemical staining but had neuroendocrine morphology, such as rosette formation and palisading. Pathological diagnoses were based on the criteria of the WHO guidelines.[16] Disease stages were classified according to the 7th edition of the Union for International Cancer Control tumor, node, metastasis classification system.[17]

The Fisher exact test was used to compare each categorical variable. Survival curves were plotted according to the Kaplan-Meier method and compared using the log-rank test. Overall survival (OS) was measured from the day of pulmonary resection to the date of death from any cause or the date on which the patient was last known to be alive. The recurrence-free survival (RFS) was measured as the interval between the date of resection and the date of recurrence diagnosis, or the date of death from any cause, or the latest date on which the patient was last known to be alive and disease-free, confirmed on the last CT before death. The Cox proportional hazards models were used to explore the effect of other clinicopathological factors to identify statistically independent prognostic factors. All tests were 2-sided, and P values $< .05$ were considered to be statistically significant. We used StatvView statistical software version 5.0 for Windows (SAS Institute Inc) for all statistical analyses.

Results

The study cohort included 119 men and 21 women, with a median age of 70 years (range, 22-85 years). The follow-up period for the patients in this study ranged from 2 to 133 months. The median follow-up time was 60 months (Table 1). Almost all the patients had a smoking history (138 patients; 99%). The median Brinkman Index was 1000 (range, 0-4160). The survival curves for the 140 patients with high-grade NEC according to the histological type are shown in Figure 1. Figure 1A shows the OS curves, and the 5-year OS rates of LCNEC and SCLC patients were 53.3% and 61.5%, respectively. There was no statistical difference in OS ($P = .30$). RFS curves are plotted in Figure 1B. The 5-year RFS rates of LCNEC and SCLC patients were 43.5% and 45.5%, respectively. There was no statistical difference in RFS ($P = .79$).

Of the 140 tumors, 59 tumors were diagnosed as SCLC. Of these, 43 were pure SCLC, and the remaining 16 were combined SCLC. Pure SCLC patients were younger than combined SCLC patients ($P = .04$). Tumor diameter was significantly larger in combined tumors ($P < .01$). There were no statistically significant differences in sex, smoking status, lymph node metastasis, lymphatic permeation, vascular invasion, or pleural invasion between pure and combined SCLC. The OS and RFS were not significantly different between the groups (OS, $P = .62$; 5-year OS, 63.2% vs. 58.8%; RFS, $P = .91$; 5-year RFS, 42.8% vs. 43.2%). Similar results were also observed when only data of stage I SCLC patients were analyzed (OS, $P = .64$; 5-year OS, 82.0% vs. 68.8%; RFS, $P = .51$; 5-year RFS, 58.6% vs. 68.8%).

Table 1 Clinicopathological Features of Neuroendocrine Carcinomas

Factor	SCLC (n = 59)	LCNEC (n = 81)
Sex, Male / Female	50 / 9	69 / 12
Age, Median (Range)	68 (22-85)	70 (22-84)
Stage		
IA / IB	23 / 9	23 / 25
IIA / IIB	9 / 6	13 / 4
III / IV	10 / 2	15 / 1
Type of Surgery		
Lobectomy	56	77
Pneumonectomy	3	4
Combination		
Pure	43	56
With LCNEC	10	–
With Ad	2	16
With Sq	1	8
With others	3	1

Abbreviations: Ad = adenocarcinoma; LCNEC = large cell neuroendocrine carcinoma; SCLC = small cell lung carcinoma; Sq = squamous cell carcinoma.

Univariate analysis was performed to explore the relationship between clinicopathological factors and OS or RFS rates at 5 years in patients with SCLC of all stages (Table 2). Lymph node metastasis ($P < .01$ in RFS and OS) and lymphatic permeation ($P = .03$ in RFS) were significantly associated with poorer prognosis. When only data of pathological stage I patients were analyzed (data not shown), no factors were statistically associated with poorer outcome.

In SCLC, we did not perform multivariate analysis because the 2 prognostic factors which were significant on univariate analysis, namely, lymph node metastasis and lymphatic permeation, were previously shown to be strongly correlated with each other.[18]

A total of 81 tumors were diagnosed as LCNEC. Of these, 56 were pure LCNEC and 25 were combined LCNEC. There were no significant differences between pure and combined LCNEC. There were no significant differences in OS and RFS between pure and combined LCNEC compared in any stage (OS, $P = .92$; 5-year OS, 53.2% vs. 58.8%; RFS, $P = .52$; 5-year RFS, 41.2% vs. 56.4%) and only in stage I tumors (OS, $P = .97$; 5-year OS, 58.8% vs. 78.8%; RFS, $P = .46$; 5-year RFS, 48.6% vs. 80.0%).

On univariate analysis of clinicopathological factors of all stages for OS and RFS (Table 3), female sex ($P = .03$ in RFS; $P = .02$ in OS), larger tumor diameter ($P = .01$ in RFS; $P = .02$ in OS), lymph node metastasis ($P < .01$ in RFS and OS), lymphatic permeation ($P < .01$ in RFS and OS), and vascular invasion ($P = .01$ in RFS; $P = .02$ in OS) were significantly associated with poorer outcome. In pathological stage I LCNEC patients, female sex ($P = .02$ in RFS and OS), larger tumor diameter ($P = .02$ in RFS; $P = .04$ in OS), and vascular invasion ($P = .03$ in RFS; $P = .04$ in OS) were significantly associated with poorer prognosis (data not shown).

The clinicopathological factors with P values $< .1$ on univariate analysis in the OS of all stages of LCNEC patients were entered into the multivariate analysis, and 2 factors were identified to be independent unfavorable predictors of survival: tumor diameter (hazard ratio [HR], 2.012; 95% confidence interval [CI], 1.045-3.891; $P = .01$), and lymphatic permeation (HR, 2.591; 95% CI, 1.355-4.951; $P < .01$).

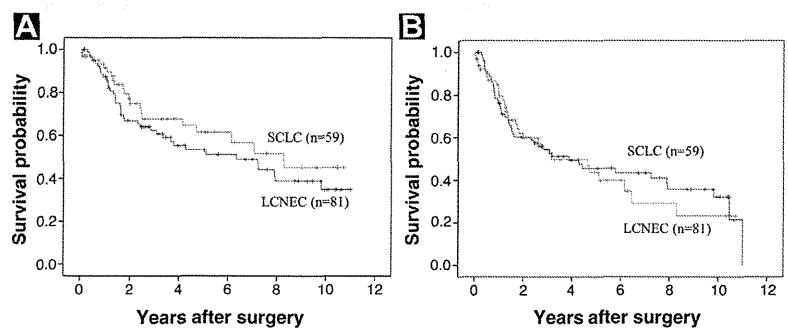
Discussion

We demonstrated that there were no clinicopathological differences between pure and combined high-grade NEC. In the current series, approximately one-third of high-grade NEC tumors were combined with other carcinoma subtypes, and the differences between pure and combined high-grade NEC remain unclear. Hage et al compared the survival of patients with combined SCLC and those with pure SCLC after surgical resection.[19] They reported that there were no significant differences in cumulative 5-year survival between the 2 SCLC subtypes of pathological stage I, II, or III tumors. OS and RFS were not significantly different between pure and combined subtypes for either SCLC or LCNEC. There have been no reports describing the difference between pure and combined SCLC or LCNEC since the histological entity of LCNEC was first introduced in the WHO classification of 1999. When we examined the differences between combined and pure subtypes in high-grade NEC in all stages or only stage I, no significant differences were observed in either OS or RFS (data not shown). Therefore, we speculate that pure and combined high-grade NEC might not have biologically different characteristics.

Although these tumors are distinctively different histologically in most cases, LCNEC and SCLC have been reported to result in similarly poor outcomes, as shown in Figure 1. Veronesi et al demonstrated that the survival rate at 5 years seemed to be influenced by age, and pathological stage in their cohort of 144 LCNEC patients.[20] In the current 81 LCNEC patients, larger tumor diameter, lymph node metastasis, lymphatic permeation, and vascular invasion were unfavorable prognostic factors. When only stage I LCNEC patients were evaluated, larger tumor diameter and vascular invasion were strong unfavorable prognostic factors. Moreover, multivariate analysis identified larger tumor diameter and lymphatic permeation to be strong prognostic factors in all stage LCNEC. The results of the current study demonstrated that these clinicopathological factors were strongly correlated with a poor outcome in LCNEC, similar to those of lung adenocarcinomas.[21,22] In contrast, in the SCLC patients in the current study, tumor diameter and vascular invasion were not significantly associated with prognosis, but nodal metastasis and lymphatic permeation were. However, none of these were identified as significant prognostic factors when only data from patients with stage I SCLC were analyzed. The International Association for the Study of Lung Cancer lung cancer staging project reported that both T and N statuses had a strong effect on survival after the resection of SCLC in 349 patients.[23] However, in the current study, neither RFS nor OS significantly differed according to tumor size. These results might suggest that tumor diameter and vascular invasion have a weaker prognostic effect than lymphatic spread in SCLC. Although patients with LCNEC and those with SCLC have similarly poor prognoses, these differences in prognostic factors might suggest LCNEC and SCLC have different biological behavior.

Sarkaria et al showed that the univariate analysis in pathological stage IB-IIIA completely resected patients receiving combination

Figure 1 Survival Curves. Overall Survival (A) and Recurrence Free Survival (B) Curves for Patients With LCNEC and SCLC of All Stages



Abbreviations: LCNEC = large cell neuroendocrine carcinoma; SCLC = small cell lung carcinoma.

Table 2 Overall Survival Rate and Recurrence Free Survival Rate in Small-Cell Lung Carcinoma Patients of All Stages and Clinicopathological Factors

Factor	n	5-years RFS (%)	*P*	5-years OS (%)	*P*
Sex			.07		.17
Male	50	50.1		41.7	
Female	9	33.3		20.8	
Age, years			.50		.83
≤70	27	49.0		59.8	
>70	32	40.8		62.3	
Smoking Status (B.I.)			.81		.18
≤1000	28	45.0		51.2	
>1000	31	44.6		68.9	
Tumor Diameter (cm)			.56		.18
Less than 3	24	48.4		71.6	
3 or more	35	40.1		53.1	
Nodal Metastasis			<.01		<.01
Yes	22	18.8		34.0	
No	37	63.6		82.2	
Lymphatic Permeation			.03		.10
Present	24	24.0		46.7	
Absent	35	61.2		70.8	
Vascular Invasion			.35		.17
Present	49	43.9		58.6	
Absent	10	34.3		66.7	
Pleural Invasion			.74		.57
Present	20	50.3		58.6	
Absent	39	40.3		57.4	

Survival curves were plotted according to the Kaplan-Meier method using a log-rank test.
Abbreviations: B.I. = Brinkman index; OS = overall survival; RFS = recurrence free survival.

platinum-based induction and (or) adjuvant chemotherapy showed a trend toward improved OS, compared with the no chemotherapy group.[24] Iyoda et al reported that patients who underwent platinum-based adjuvant chemotherapy had a significantly lower rate of tumor recurrence and a higher rate of disease-free survival than those who had nonplatinum-based adjuvant chemotherapy or no adjuvant chemotherapy in a series of 72 resected LCNEC patients. They also demonstrated that platinum-based adjuvant chemotherapy was an independent prognostic factor in multivariate survival analyses.[25] Patients with advanced LCNEC might benefit from platinum-based adjuvant chemotherapy after surgical resection.[5]

Table 3 Overall Survival Rate and Recurrence Free Survival Rate in Large-Cell Neuroendocrine Carcinoma Patients of All Stages and Clinicopathological Factors

Factor	n	5-years RFS (%)	*P*	5-years OS (%)	*P*
Sex			.03		.02
Male	69	47.7		57.3	
Female	12	25.0		22.2	
Age, years			.29		.27
≤70	41	41.1		51.2	
>70	40	47.6		53.4	
Smoking Status (B.I.)			.85		.85
≤1000	41	46.9		55.9	
>1000	40	41.9		48.9	
Tumor Diameter (cm)			.01		.02
<3	45	39.0		43.8	
≥3	36	53.1		63.1	
Nodal Metastasis			<.01		<.01
Yes	24	16.7		24.9	
No	57	54.7		62.5	
Lymphatic Permeation			<.01		<.01
Present	34	26.1		33.5	
Absent	47	57.4		65.8	
Vascular Invasion			.01		.02
Present	65	38.1		45.1	
Absent	16	69.6		78.0	
Pleural Invasion			.35		.28
Present	32	42.0		45.2	
Absent	49	45.0		55.8	

Survival curves were plotted according to the Kaplan-Meier method using a log-rank test.
Abbreviations: B.I. = Brinkman index; OS = overall survival; RFS = recurrence free survival.

A limitation of this retrospective study was that only surgical cases were included. This was intended to ensure accurate recording of the histopathological characteristics based on sufficient specimens and therefore, advanced, unresectable tumors were not included. Most SCLC patients are not candidates for surgical resection because of local/systemic spread. The SCLC tumors in the present series might not be representative of typical SCLC, which might have a more aggressive nature. Although it is evidently difficult to obtain sufficient specimens using only biopsy samples in nonsurgical patients and to perform a thorough histopathological evaluation with immunohistochemical staining, histological analysis of tumors from patients with advanced diseases should be included in future studies. Furthermore, because the patient number was small, a negative finding might well be because the study is underpowered to identify a survival difference.

Conclusion

Despite these limitations, there were no apparent differences in the clinicopathological characteristics and outcomes between pure and combined subtypes in either SCLC or LCNEC. Therefore, we speculate that pure and combined high grade NECs might not have biologically different characteristics. Furthermore in SCLC, lymphatic involvement was an important unfavorable prognostic factor. SCLC patients without lymphatic involvement might be expected to have a good prognosis. In general, resectable high-grade NEC is a rare entity, and therefore it is very difficult to study. To elucidate the malignant behavior of high-grade NEC, larger multiinstitution clinicopathological studies are needed to be performed prospectively, leading to more useful therapeutic options.

Clinical Practice Points

- We reviewed the clinical data of 140 resected high-grade NEC patients and analyzed the clinicopathological features in relation to their survival. There were no apparent differences in biological behavior between pure and combined subtypes in either LCNEC or SCLC.
- We suggested that the patients diagnosed to have LCNEC or SCLC, regardless of pure or combined type, should be treated equally.
- We demonstrated that unfavorable prognostic factors were different in LCNEC and SCLC. The biological behavior of LCNEC was very similar to that of other lung carcinomas.
- In SCLC, lymphatic involvement was an important unfavorable prognostic factor. The tumor diameter, and vascular and pleural invasion were not statistically correlated with patients' poor outcome. Patients with SCLC without lymphatic involvement might be expected to have a good prognosis.

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Disclosure

The authors have stated that they have no conflicts of interest.

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Identification of intravascular tumor microenvironment features predicting the recurrence of pathological stage I lung adenocarcinoma

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Histological vascular invasion (VI) by tumors is reportedly a risk factor influencing recurrence or survival after surgical treatment; however, few studies have evaluated which VI features affect recurrence or survival. The objective of this study was to evaluate how VI features affect recurrence in lung adenocarcinoma patients. We selected 106 patients with pathological stage I lung adenocarcinoma who showed VI and examined the properties of intravascular tumors associated with recurrence. First we investigated the relationship between the frequency of VI in a histological cross-section and the incidence of recurrence; however, a significant impact was not observed. Microscopic examination revealed the intravascular tumors were composed of not only cancer cells but also non-cancerous cells. To examine whether the characteristics of intravascular cancer cells and/or non-cancerous cells have prognostic value, we examined the expression levels of epithelial–mesenchymal transition-related markers in cancer cells and the numbers of infiltrating non-cancerous cells, including macrophages, endothelial cells, and fibroblasts. High levels of E-cadherin expression in the intravascular cancer cells were significant predictors of recurrence ($P = 0.004$), whereas the expressions of CD44, CD44 variant 6, and vimentin were not. Large numbers of intravascular CD204(+) macrophages ($P = 0.016$), CD34(+) microvessels ($P = 0.007$), and α-smooth muscle actin (+) fibroblasts ($P = 0.033$) were also significant predictors of recurrence. Our results indicated VI with abundant stromal cell infiltrates might be a predictor of recurrence and suggested the tumor microenvironment created by cancer cells and stromal cells within the blood vessel may play an important role during the metastatic process. (*Cancer Sci* 2013; 104: 1262–1269)

The cause of death in most cancer patients is the development of metastases from the primary tumor. The metastatic process includes various complex steps. The process starts with the separation of cancer cells from the primary lesion, followed by the permeation of these cells into vessels. In the permeated vessels, the cancer cells overcome apoptosis (also known as anoikis), proliferate, and then transmigrate to the metastatic site.

Vascular invasion represents the early phase of metastasis.[1–3] Therefore, the presence of histological intratumoral VI has been reported to be a predictor of recurrence and metastasis in surgical cases with various types of cancer.[4–6] In fact, VI by testicular germ cell tumors qualifies as the local spread of tumors.[7,8] Testicular tumors localized to the testis and epididymis without VI are classified as T1, whereas they are classified as T2 when VI is present. Although VI has been reported to be a strong prognostic factor in NSCLC, the impact of VI on the revised TNM classification of NSCLC remains to be fully assessed.

Nowadays, tumor tissue is known to be composed of variable numbers of cancer cells and stromal cells, such as macrophages, endothelial cells, and fibroblasts, and cancer cells are known to interact with these surrounding stromal cells, producing a specific microenvironment that is capable of influencing tumor progression.[9] Furthermore, recent studies have reported that the intravessel microenvironment has significant effects on metastasis as well as the disease at the primary site. Matsumura *et al.*[10] reported that intralymphatic cancer cell and stromal cell phenotypes are susceptible to lymphogenic metastasis, suggesting that lymphogenic metastasis may be affected by the intralymphatic microenvironment created by these cells.

Lung cancer is the leading cause of cancer-related deaths worldwide,[11] and NSCLC accounts for the majority of lung cancers.[12] Adenocarcinoma is the most frequent histological type of NSCLC, and its incidence is increasing in most countries.[13] In Japan, adenocarcinoma is the most common histological type of resected lung cancer, accounting for more than 60% of all cases.[14] However, although surgical resection is considered to be the most effective therapy for patients with stage I adenocarcinoma, approximately 17.3% of patients develop recurrences.[15] Several studies have reported that the presence of VI has a significant impact on the recurrence of lung adenocarcinoma and the survival of patients after surgical treatment. However, few studies have investigated which VI features influence recurrence and/or survival. This issue needs to be examined to clarify the mechanisms underlying the early phase of metastasis.

The objective of the present study was to evaluate how VI features affect recurrence in lung adenocarcinoma. We therefore examined the frequencies and characteristics of intratumoral VI features and their correlations with recurrence.

Materials and Methods

Patient selection. A total of 1099 consecutive patients with pathological stage I primary lung adenocarcinoma underwent surgical resection between January 1995 and December 2007 at the National Cancer Center Hospital East, Chiba, Japan. All the surgical specimens were collected and analyzed after receiving the approval of the Ethics Committee of our institution. No patient consent was required as the research was a retrospective chart review and no personally identifiable information was included.

To examine how VI features affect recurrence, we selected 106 patients with VI excluding patients with pleural invasion (another important predictor of recurrence) from the 1099

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