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Reasonable Extent of Lymph Node Dissection in Intentional Segmentectomy for Small-Sized Peripheral Non–Small-Cell Lung Cancer

From the Clinicopathological Findings of Patients Who Underwent Lobectomy with Systematic Lymph Node Dissection

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Introduction: Currently, randomized clinical trials to evaluate segmentectomy compared with lobectomy for peripheral cT1aN0M0 non–small-cell lung cancer (NSCLC) are ongoing. During segmentectomy, some lobar-segmental lymph nodes (LSNs) can be difficult to resect for anatomical reasons. The purpose of this study was to clarify the reasonable extent of dissection during intentional segmentectomy for peripheral cT1aN0M0 NSCLC.

Methods: We reviewed the records of patients who underwent lobectomies and systematic lymph node dissections for cT1aN0M0 NSCLC from 1992 to 2009. Among them, a total of 307 patients whose primary nodule was located in the outer third peripheral lung field on thin-section computed tomography (TSCT), and who could be candidates for intentional segmentectomy were enrolled in this study. We analyzed the clinical and radiological factors, which may predict nodal metastasis, and the distribution patterns of lymph node metastases. In particular, we set out to evaluate the specific LSNs, which are difficult to resect on segmentectomy (isolated LSNs [iLSNs]).

Results: Of all patients, 34 (11%) had lymph node metastases (pN1: 9, pN2: 25). The median tumor sizes and tumor disappearance rates (TDRs) on TSCT were significantly larger and lower, respectively, compared with those of the remaining 273 node-negative patients. All 34 node-positive patients had a solid-dominant component on TSCT (TDR < 0.25). Of these, nine patients ($n = 5$, station 11, $n = 4$, station 13) were iLSN positive, but all of them also had metastases to station 12 or mediastinal lymph nodes. No patients had solitary metastasis in iLSNs.

Conclusions: The reasonable extent of dissection for intentional segmentectomy for small (≤ 2 cm) peripheral NSCLC includes LSNs in the segments with tumors, and the hilar and mediastinal nodes. It may not be necessary to examine iLSNs. Systematic lymph node dissection might not be necessary for tumors with ground grass opacity on TSCT (TDR ≥ 0.25).

Key Words: Lung cancer, Intentional segmentectomy, Lymph node dissection.

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Since the randomized phase III trial conducted by the Lung Cancer Study Group demonstrated in 1995[1] a threefold increase in local recurrence rate (17.2% versus 6.4%) and worse survival among patients who underwent sublobar resection, the standard surgical procedure for clinical stage IA non–small-cell lung cancer (NSCLC) has been considered lobectomy with systematic lymph node dissection. However, the widespread use of computed tomography (CT) scans has increased the number of patients with small-sized NSCLC of 2 cm or less. Recent studies have demonstrated that within clinical stage IA, patients with T1a tumors of less than 2 cm have a better survival rate than those with T1b tumors of between 2 and 3 cm. It has been hypothesized that T1a tumors might be successfully treated by sublobar resection. Several retrospective studies have demonstrated that segmentectomy with lymph node dissection in sublobar resection achieved excellent survival comparable to lobectomy for peripherally located T1a tumors of 2 cm or less in size.[2–5] Currently, in Japan (JCOG0802/WJOG4607L) and in the United States (CALGB-140503), randomized prospective clinical trials are ongoing to evaluate segmentectomy compared with lobectomy for peripheral cT1aN0M0 NSCLC.[6,7] Intentional segmentectomy should be performed only for small-sized, clinical, and pathological N0 tumors in trial settings, and dissected lymph nodes are required to be carefully examined to confirm pN0 status. When positive lymph nodes are found intraoperatively, it is stipulated to be converted to lobectomy in these trials.

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However, the appropriate extent of lymph node dissection, particularly in the lobar and segmental lymph node (LSN) areas remains unclear. LSNs other than those in the resected segmental bronchi are technically difficult for segmentectomy. For example, station 11s (the interlobar node located between the upper lobe bronchus and bronchus intermedius on the right) is easy to resect during posterior (S^2) segmentectomy of the right upper lobe, but is technically demanding during anterior (S^3) segmentectomy because station 11s is in the dorsal area of the root of the right upper bronchus and is distal from B3 (Fig. 1). For the same reason, the dissection of station 13 (segmental lymph node) in nonresected segments is also difficult, as shown in Figure 1. It has not yet been clarified which LSNs should be included in the dissection area, or whether LSNs in the nonresected segments should be dissected during segmentectomy. In the current study, we examined patterns of lymph node metastases, particularly those of LSN stations in cases of peripheral cT1aN0M0 NSCLC, to clarify the appropriate nodal dissection extent during anatomical segmentectomy by retrospectively examining the data of patients who underwent lobectomy and systematic lymph node dissection.

PATIENTS AND METHODS

Patients

Between 1992 and 2009, there were 1167 consecutive patients who underwent complete resection of a clinical T1aN0M0 lung cancer. We reviewed their clinicopathologic records and selected for this retrospective study 307 patients who underwent lobectomy and systematic lymph node dissection for peripheral cT1aN0M0 NSCLC (Fig. 2). The primary tumors of these patients were located in the outer third of the peripheral lung field on thin-section computed tomography (TSCT), and were possible candidates for the ongoing randomized trials of intentional segmentectomy. The following patients were excluded because of unsuitable clinicopathologic conditions for intentional segmentectomy (Fig. 2): patients who underwent procedures other than lobectomy or systematic lymph node dissection ($n = 368$), patients with small-cell lung cancer, large-cell neuroendocrine carcinoma, or carcinoids ($n = 55$), patients with a tumor located in the right middle lobe ($n = 104$), and patients with multiple primary lung cancers ($n = 66$). As their radiological findings were inconsistent, patients with nonperipheral cancer ($n = 162$), cancer with predominant ground glass opacity (GGO) on TSCT ($n = 10$), and patients without immediate preoperative TSCT ($n = 95$) were also excluded. Data collection and analyses were approved and the need to obtain written informed consent from each patient was waived by the Institutional Review Board in December 2010. We analyzed the prevalence and patterns of lymph node metastases of all patients and identified the clinical and radiological factors that may predict nodal metastases.

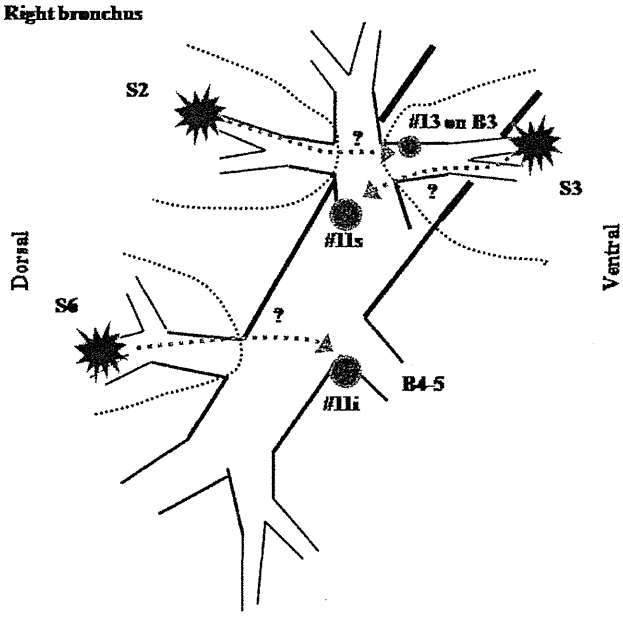

FIGURE 1. Diagram of an iLSN in the right bronchus. If the tumor was located in the superior or anterior segment of the right upper lobe (S^1 or S^3), station 11s was categorized as iLSN. The node located in a different segmental bronchus was also classified as iLSN. iLSN, isolated lobar-segmental lymph node.

Radiological Evaluation of the Primary Tumor

Contrast-enhanced CT was performed to evaluate the entire lung for preoperative staging. In addition, the main tumor was also evaluated by TSCT with 1 to 3 mm collimation to measure the extent of GGO. TSCT findings were evaluated by two observers (YM and TH), who were blinded to patient identity. The observers measured the preoperative tumor size and tumor shadow disappearance rate (TDR) of all 307 consecutive patients. TDR was calculated using the following formula: TDR = 1 − (maximum tumor size on mediastinal window/maximum tumor size on lung window).[8] Evaluation discrepancy between the observers was resolved by consensus.

Histological Evaluation of the Primary Tumor and Lymph Nodes

The surgical specimens examined were fixed in 10% formalin or 100% methyl alcohol. The specimens were then sliced at the maximum dimension of the primary tumor and all sections were embedded in paraffin. Pathologists identified all peripheral subsegmental bronchi and surrounding lymph nodes. We also obtained specimens from subsegmental bronchi and lymph nodes from the section without lesions, slices of which were also embedded in paraffin. All serial 4-μm sections were stained with hematoxylin and eosin. In this study, station 11, 12, and 13 lymph nodes were focused and named as LSNs. We further classified them into two categories based on the anatomical relationship with the area of involved segmental bronchus: the LSN was adjacent to the involved segmental bronchus (adjacent LSN, aLSN) or the LSN was located isolated from the involved segmental bronchus (isolated LSN, iLSN). The aLSN is the lymph node located proximal to the involved segmental bronchus and it can be easily resected during segmentectomy. The iLSN is the lymph node located distal to the resected segmental bronchus and it can be technically challenging to resect. Station 11, if located in the same lobe

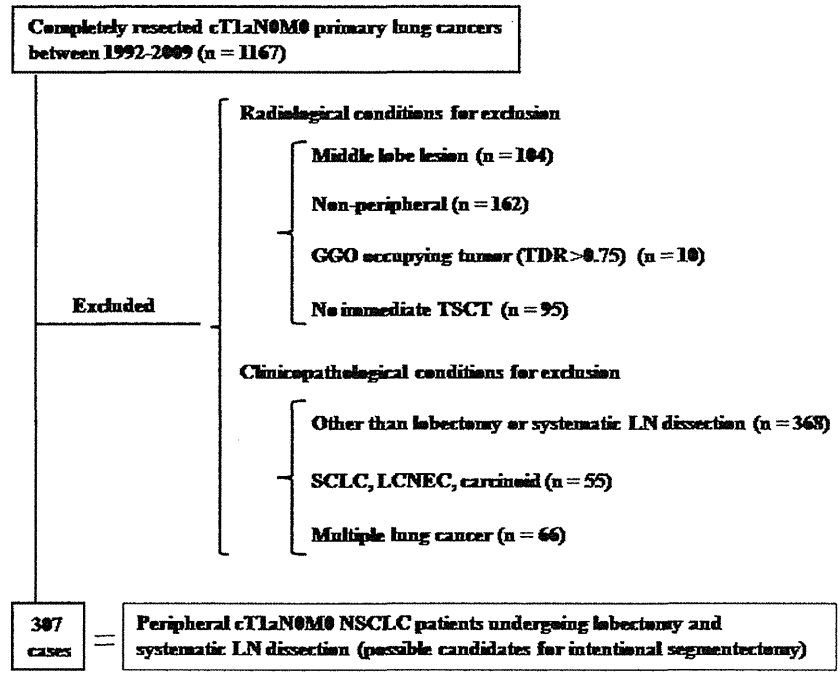

FIGURE 2. Patient characteristics. GGO, ground grass opacity; LCNEC, large-cell neuroendrocrine carcinoma; LN, lymph node; NSCLC, nonsmall-cell lung cancer; SCLC, small-cell lung cancer; TSCT, thin-slice computed tomography;

as the primary tumor, is classified as either aLSN or iLSN depending on the location (segment) of the tumor. As shown in Figure 1, if the primary tumor is in the posterior segment of the right upper lobe (S^2), station 11s is defined as aLSN. In contrast, if the tumor is located in the superior or anterior segment of the right upper lobe (S^1 or S^3), station 11s is categorized as iLSN. Station 12, if located in the same lobe as the primary tumor, is classified as aLSN. For station 13, the node located in the same segment as the primary tumor (ss13) is classified as aLSN. The node located in a different segmental bronchus (ds13) was classified in the iLSN. In the current series, if the tumor was located over two segments, the definition of either aLSN or iLSN was made by the main segment. Histological typing, pathological stage, and nodal station were determined based on the Tumor Node Metastasis classification and nodal definition of the Union for International Cancer Control.[9–11]

Statistical Analyses

The Mann–Whitney U test or χ^2 test was used to test differences in the clinicopathologic characteristics (age, sex, preoperative serum carcinoembryonic antigen level, tumor size, TDR, histologic type, and pN status) between two groups for statistical significance. All p values reported are two-sided, and the significance level was set at less than 0.05. The analyses were performed with the SPSS 11.0 statistical software program (Dr. SPSS II for Windows, standard version 11.0, SPSS Inc., Chicago, IL).

RESULTS

Clinicopathologic Characteristics and Prevalence of Lymph Node Metastases

The clinicopathologic characteristics of all 307 patients are shown in Table 1. The median age was 63 years (range, 32–83), and the median preoperative tumor size on TSCT was 1.7 cm (range, 0.8–2.0). Adenocarcinoma was the most common histologic type, observed in 271 cases (88%), and the predominant subtypes included 44 minimally invasive adenocarcinomas, 94 papillary subtypes, 81 lepidic growth subtypes, 36 solid subtypes, 15 acinar subtypes, and one micropapillay subtype according to the newly introduced adenocarcinoma classification. A total of 34 patients (11%) had lymph node metastases (pN1: 9, pN2: 25). Table 2 shows the correlations between the clinicopathologic factors and pathologic nodal status. Compared with the pN0 tumors (n = 273), pN1–2 tumors had a significantly larger median preoperative tumor size (1.6 cm; range, 0.8–2.0 versus 1.8 cm; range, 1.4–2.0, p = 0.03). However, with TDR there were no patients with node metastases among the patients with TDR of 0.25 or more, so we chose 0.25 as the cutoff value of the TDR in the present study. When the cutoff value of the TDR was set at 0.25,

TABLE 1. Patient Characteristics

	$n = 307$
Median age, yrs (range)	63 (32–83)
Male sex	162 (53%)
Serum CEA (ng/mL) > 5.0	76 (25%)
Median preoperative tumor size, cm (range)	1.7 (0.8–2.0)
TDR	
< 0.25	197 (64%)
0.25–0.75[a]	110 (36%)
Histology	
Adenocarcinoma	271 (88%)
Squamous cell carcinoma	26 (8%)
Large-cell carcinoma	6 (2%)
Pleomorphic carcinoma	2 (1%)
Adenosquamous carcinoma	2 (1%)
pN status	
pN0	273 (89%)
pN1	9 (3%)
pN2	25 (8%)

$$\text{TDR} = 1 - \frac{\text{maximum diameter on mediastonal window}}{\text{maximum diameter on lung window}}$$

[a]Cancers with TDR > 0.75 were excluded from this study as they were considered to be non- or minimally invasive carcinomas.

CEA, preoperative carcinoembryonic antigen level; TDR, tumor disappearance rate.

TABLE 2. Correlation between Clinicopathologic Factors and Pathologic N Status

Factors	pN0 ($n = 273$)	pN1/2 ($n = 34$)	p
Median age (yrs)	63	59	0.31
Male sex ($n = 162$)	143 (88)	19 (12)	0.60
Median preoperative tumor size	1.6	1.8	0.03
Serum CEA (ng/mL)			
> 5.0 ($n = 76$)	64 (84)	12 (16)	0.13
≤ 5.0 ($n = 231$)	209 (90)	22 (10)	
TDR			
< 0.25 ($n = 197$)	163 (83)	34 (17)	< 0.01
≥ 0.25 ($n = 110$)	110 (100)	0	
Histology			0.98
Adenocarcinoma ($n = 271$)	241 (89)	30 (11)	
Nonadenocarcinoma ($n = 36$)	32 (89)	4 (11)	

Numbers in parentheses are percentages.

CEA, preoperative carcinoembryonic antigen level; TDR, tumor disappearance rate.

the difference in TDR was statistically significant between the pN0 and pN1–2 groups ($p < 0.01$). Among 197 patients with a TDR less than 0.25, 34 patients (17%) had positive lymph node findings (N1: 9, N2: 25). In contrast, all the 110 patients with a TDR of 0.25 or more were node negative. The histological types for all patients with pN1–2 disease were adenocarcinoma, except one case of squamous cell carcinoma. The differences in age, sex, or histological patterns were not statistically significant between the pN0 and pN1–2 groups.

Patterns of Lymph Node Metastases

The patterns of lymph node metastasis in LSNs are shown in Figure 3. Of the 34 pN1–2 patients, metastases in LSNs (stations 11, 12, and 13) were observed in 22 patients (7% of all patients, and 11% of the patients with a TDR of < 0.25). The metastatic lymph node stations of 22 patients with LSN involvement are described in Table 3. Among them,

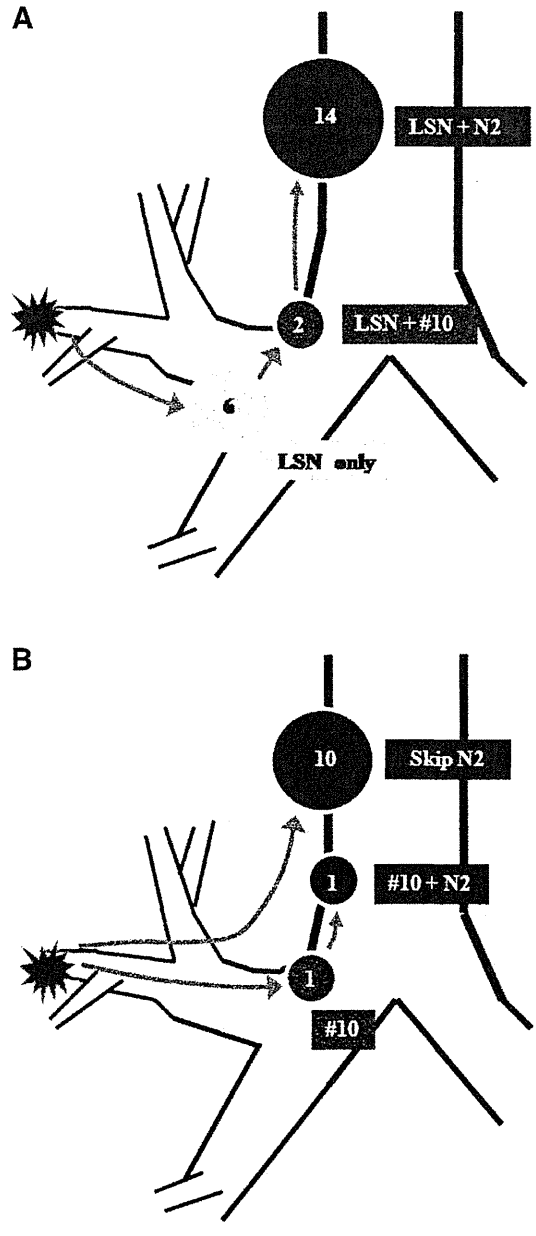

FIGURE 3. Distribution patterns of lymph node metastases with LSN node involvement. *A*, Among the patients with LSN metastases ($n = 22$), 6 patients had metastases in the LSN only. *B*, Among the patients without LSN metastases ($n = 12$), 10 patients had metastases in the mediastinal stations only (skip N2). LSN, lobar-segmental lymph node.

TABLE 3. Metastatic Lymph Node Stations of the Patients with LSN Metastases

Pt	Lobe	Involved Segment	Hist.	Mediastinal Area			Non-LSN	LSN[a]
				Upper	Subcarinal	Lower		
1	RU	S1	Ad	4			10	12u, ss13, 14
2	RU	S1	Ad	4				ss13
3	RU	S2	Ad					12u, **ds13 (B3)**
4	RU	S2	Ad	4				12u
5	RU	S2	Ad					ss13
6	RU	S3	Ad	2, 3a, 4				**11s**, 12u
7	RL	S6	Ad		7			**11i**, 12l, ss13
8	RL	S6	Ad					12l
9	RL	S8	Ad					11i
10	LU	S1+2	Ad	4, 5				12u
11	LU	S1+2	Ad				10	ss13
12	LU	S1+2	Ad					12u, **ds13 (B4–5)**
13	LU	S3	Ad	6	7		10	**11**, 12u
14	LU	S3	Ad				10	ss13
15	LU	S4	La	5				**ds13 (B3)**
16	LU	S4	Ad	4, 5				12u
17	LU	S4	Ad					12u
18	LL	S6	Ad		7			**11**
19	LL	S8	Ad	6	7			11, 12l
20	LL	S9	Ad		7			**ds13**
21	LL	S10	Pleo		7			**11**
22	LL	S10	Sq		7			12l

[a]LSNs in bold correspond to iLSN for each patient.

Pt, patient; RU, right upper lobe; RL, right lower lobe; LU, left upper lobe; LL, left lower lobe; Hist, histology; Ad, adenocarcinoma; La, large-cell carcinoma; Pleo, pleomorphic carcinoma; Sq, squamous cell carcinoma; LN, lymph node; LSN, lobar-segmental lymph node.

six patients (2% of all patients, and 3% of patients with a TDR of < 0.25) had LNS metastases only. In the other 16 patients, metastases were observed in not only the LSNs but also in other areas; namely, the trachea-bronchial station (station 10) (n = 2) and mediastinal stations (n = 14). Among 12 patients without LSN metastases, 10 patients had metastases limited to mediastinal stations (skip N2, Fig. 3*B*).

Figure 4*A* and *B* shows the details of six patients with metastases in the LSNs only (patients 3, 5, 8, 9, 12, and 17; Table 3). All patients were found to have adenocarcinoma on histologic diagnosis and metastases in the aLSN. Of these six patients, two patients (3 and 12) had metastases in the iLSN (ds13), which were technically challenging to resect. However, both patients also had metastases in station 12 (aLSN). No patient had solitary iLSN metastasis.

There were nine patients with iLSN metastases (3% of all patients, 26% of pN1–2 patients). Among them, five patients (patients 6, 7, 13, 18, and 21; Table 3) had metastasis in station 11 in the iLSN and the other four patients (patients 3, 12, 15, and 20) had ds13 metastases. However, all nine patients also had metastases in station 12 or other mediastinal stations. No patients had solitary metastasis in iLSNs.

DISCUSSION

Radical sublobar resection of anatomical segmentectomy has been recognized as a treatment option for small-sized peripheral NSCLC, and randomized clinical trials to evaluate segmentectomy compared with lobectomy for peripheral cT1aN0M0 NSCLC are ongoing in Japan and the United States.[6,7] However, it is not unusual to encounter lymph node metastasis in small-sized peripheral NSCLC, particularly in tumors with a predominantly solid component on CT. As shown in Table 4, it has been reported that 10% to 15% of patients with peripheral cT1aN0M0 NSCLC had lymph node metastases.[2,5,12–15] Therefore, even during intentional anatomical segmentectomy, systematic lymph node dissection is also recommended. In segmentectomy, the mediastinal and hilar nodes as well as aLSNs could be dissected to a similar extent to lobectomy, while dissection of iLSN is technically challenging. It remains unknown whether iLSNs should be included in the dissection area during anatomical segmentectomy.

The result in the present study demonstrated that 34 of 307 patients (11%) with peripheral cT1aN0M0 NSCLC had lymph node metastases. There were 22 patients (7%) with LSN involvement and nine (3%) with iLSN metastases. All patients with iLSN metastases had also metastases in the aLSN and other areas, but there were no patients who had solitary iLSN metastases. These results indicate that if mediastinal and hilar nodes and aLSNs are intraoperatively negative, anatomical segmentectomy of complete resection can be performed without missing either metastatic lymph nodes or opportunity to convert the procedure to lobectomy. Several researchers have

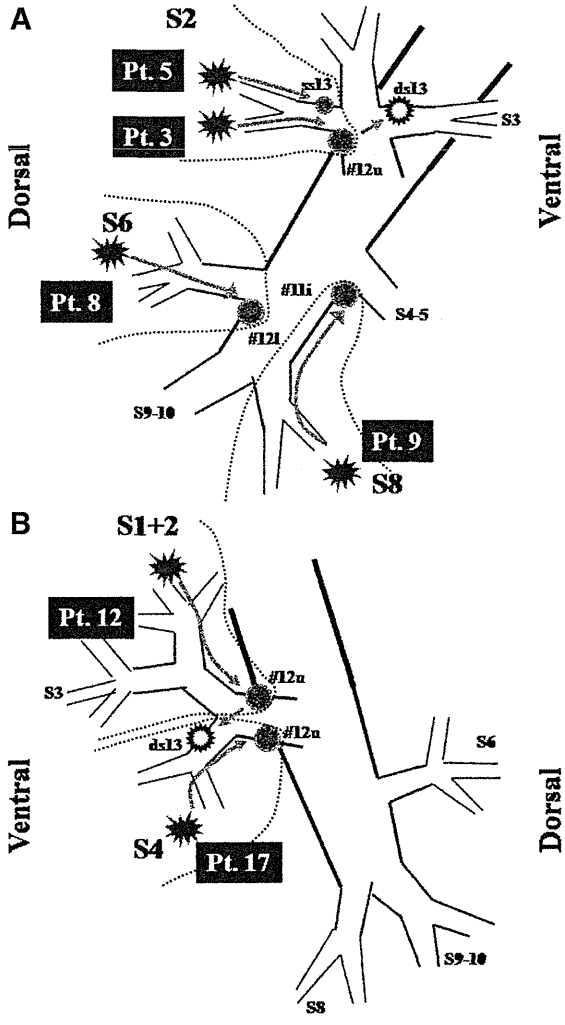

FIGURE 4. The distribution pattern of 6 patients with metastases to the LSN only. A represents the right lung and B represents the left lung. No patients had solitary metastasis in isolated LSNs. LSN, lobar-segmental lymph node; ds13, different segmental station 13 node; ss13, same segmental station 13 node.

recommended sampling both ss13 and ds13 lymph nodes during segmentectomy.[16,17] Nomori et al.[16] performed sentinel node (SN) mapping for 94 cT1N0M0 NSCLC patients who underwent segmentectomy using ^{99m}Tc-phytate, and indicated SNs not only at ss13 (termed the node in the resected segment) but also at ds13 (termed the node in the nonresected segment) in 12 of 42 patients (29%). Nodes ds13 as SNs were significantly more frequently identified in patients with tumors located in the anterior segments than in patients with tumors located in the posterior segments (47% versus 17%, $p = 0.04$). Therefore, the authors recommended radical dissection, including in ds13, especially for tumors located in the anterior segments. However, in their report, none of the 94 patients had evidence of metastasis to ds13 after radical segmental lymph node dissection. Yamanaka et al.[17]

TABLE 4. Previous Reports about Lymph Node Metastases of cT1aN0M0 NSCLC

Author	*n*	Tumor Location	Ad (%)	Procedure L	S	W	LN Mets (%)
Koike[5]	268	Peripheral	209 (89)[a]	192	62	14	20 (7)
Ikeda[13]	159	NM	159 (100)	112	27	20	15 (9)
Okada[2]	567	NM	505 (89)	262	305	0	67 (12)[b]
Inoue[14]	118	NM	97 (82)	90	23	5	15 (13)
Mattioli[15]	92	NM	59 (64)	46	46	0	3 (3)
Gomez-Caro[12]	58	NM	NM		NM[c]		7 (12)[b]
Present study	307	Peripheral	271 (88)	307	0	0	34 (11)

[a]Their histological types were described only in pT1aN0M0 patients ($n = 233$).
[b]The number and percentage of pathological stage II–III disease.
[c]The procedures were just described as "anatomic pulmonary resection plus systematic mediastinal dissection" in this report.
NM, not mentioned; LN, lymph node; Ad, adenocarcinoma; L, lobectomy; S, segmentectomy; W, wedge resection.

examined the patterns of lymph node metastases in 94 patients who had undergone standard lobectomy for peripheral cT1N0M0 NSCLC. Their series included 40 patients with tumors of 2 cm or less (cT1a), and solitary metastasis to nonprimary-tumor–bearing segments was identified in only one patient with metastasis in ds13. The dissection of lymph nodes that are isolated from the involved segmental bronchus is technically challenging in segmentectomy and may cause injury to the preserved segmental bronchus, vessels, or parenchyma. Considering the present results and previous works, we conclude that it may not be necessary to examine the iLSN, including ds13 for all segments and station 11 for certain specific segments. For example, as described earlier, if the tumor is located in the superior or anterior segment of the right upper lobe (S^1 or S^3), it may not be necessary to examine station 11s.

Our results demonstrated the extent of lymph node dissection during intentional segmentectomy, whereas it's still unclear whether all peripheral cT1aN0M0 NSCLCs need the dissection described above. In this study, none of the 110 patients with a tumor of TDR of 0.25 or more on preoperative TSCT had lymph node metastasis. Several studies have been conducted to clarify the relationship between TSCT findings and lymph node metastases in small peripheral lung cancer. Shimada et al.[18] from our institution, investigated the TSCT findings of 363 patients with peripheral cT1aN0M0 lung cancer or suspected lesions on CT. They reported that 77 of 78 patients (99%) with a tumor of TDR of 0.5 or more had noninvasive cancer, but without lymph node metastasis, vascular invasion, or pleural invasion. In the current study, the tumors with the TDR less than 0.5 contained one of the pathologically invasive characteristics mentioned above, but all tumors had pathologic node negative disease when the TDR was 0.25 or more. Inoue et al.[14] described patterns of lymph node metastases in 118 cT1aN0M0 NSCLC patients with predominantly solid (GGO area < 50%) components on TSCT. Lymph node metastases were found in 15 of 83 patients (18%) with a pure solid lesion, but none were observed in the patients with a GGO component. Although the current study was retrospectively conducted in a single institution, the present results

suggest that systematic lymph node dissection other than of iLSNs, which are necessarily removed during anatomical segmentectomy, may not be necessary for peripheral cT1aN0M0 NSCLC with GGO components on TSCT. Further studies should be conducted to establish universal radiological definitions to predict node-negative disease in small-sized peripheral NSCLC.

The present study has some limitations. It is a retrospective study, and the number of the node-positive patients was relatively small ($n = 34$) although the total number of the patients with peripheral cT1aN0M0 NSCLC enrolled in this study ($n = 307$) was one of the largest in a single institution. Further prospective studies will be needed to confirm our results in multi-institutions.

In conclusion, the proper extent of lymph node dison in intentional segmentectomy includes mediastinal and hilar nodes, as well as aLSNs for peripheral cT1aN0M0 NSCLCs. Careful inspection of these nodes using intraoperative frozen sections may enable those patients with tumors harboring occult nodal metastases to undergo standard lobectomy, if indicated. It may not be necessary to examine segmental nodes located at the iLSNs. For those tumors with GGO components (TDR $\geq$ 0.25) on TSCT, systematic lymph node inspection may not be necessary. Further studies will be needed to confirm our results in large-scale, multi-institutional, prospective studies.

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Long-Term Outcomes of 50 Cases of Limited-Resection Trial for Pulmonary Ground-Glass Opacity Nodules

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Introduction: From 1998 to 2002, we performed a trial of prospective limited resection for pulmonary ground-glass opacity lesions 2 cm or smaller. This is the second report on the long-term outcome.
Methods: The enrollment criteria of the trial were as follows: pulmonary peripheral nodule less than 2 cm, diagnosis or suspected diagnosis of clinical T1N0M0 carcinoma with ground-glass opacity and lack of evident pleural indentations or vascular convergence on high-resolution computed tomography. Limited-resection (wedge or segment) specimens were intraoperatively examined by frozen section. If the nodule was confirmed as Noguchi type A or B with a resection margin of greater than 1 cm, the incision was sutured and the patient followed up. The median surveillance period was 10 years.
Results: In a total of 50 enrolled participants, there were two Noguchi type A, 23 type B and 15 type C adenocarcinomas; five atypical adenomatous hyperplasias, four fibroses, and one granuloma. Although there were no patients with recurrence within the first 5 years, in four patients who underwent limited-resection pulmonary adenocarcinoma developed more than 5 years after the initial resection, of either cut-end recurrence or metachronous primary disease.
Conclusions: Of 26 patients who underwent limited resection, adenocarcinoma developed in four after more than 5 years. These were possibly cut-end recurrences. We concluded that 5 years is not a sufficient period for follow-up, and that limited resection should still be done only in a trial setting, even for small ground-glass opacity lesions.

Key Words: Lung cancer, Adenocarcinoma, Limited resection, Ground-glass opacity, Noguchi classification.

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In 2005, we reported the study design, methods, and preliminary results of our previous prospective trial on limited resection for pulmonary ground-glass opacity (GGO) nodules at the median follow-up point of 50 months.[1] At that time, 26 patients who had undergone limited resection (two for Noguchi type A, 23 for type B, and one for type C adenocarcinomas)[2] were free from recurrence. In those 26 patients, Noguchi type A and B lesions seemed to be in situ, noninvasive carcinomas, and we speculated that limited resection could be curative surgery for these diseases. In the trial, 14 patients with Noguchi type C lesions underwent lobectomy and lymph node dissection, and no nodal involvement, lymphatic permeation, or vascular invasion was observed. Therefore, we speculated that even patients with Noguchi type C lesions in our trial could also have been curatively treated by limited resection.

However, during continued long-term follow-up, in four of the 26 patients who underwent limited resection, pulmonary adenocarcinoma developed in areas surrounding the initial limited-resection cut-end staples more than 5 years after the initial surgery. We speculated that these might have been cut-end recurrences. We previously reported the details of three of these four patients in 2010 and warned of the possibility of late recurrence after limited resection, even for small GGO lesions.[3] With the median follow-up period of the patients enrolled in this trial having reached 10 years, we set out to determine the long-term recurrence rate of small GGO lung adenocarcinomas after limited resection.

PATIENTS AND METHODS

Patients

From 1998 to 2002, we enrolled 50 patients in this study.[1] Enrollment criteria were: tumor less than 2 cm in diameter, diagnosed as or suspected to be a clinical T1N0M0 carcinoma[4] in the lung periphery on computed tomography (CT); high-resolution CT findings suggestive of a Noguchi type A or B adenocarcinoma (i.e., GGO and lack of evident pleural indentation or vascular convergence). Patients with a history of malignancy within the previous 5 years and those who were not indicated for lobectomy and systematic lymph node dissection were excluded. Written informed consent was obtained from each participant. The study protocol was reviewed by the Institutional Review Board of the National Cancer Center Hospital East (Chiba, Japan) and was approved in July 1998.

We performed limited resection, wedge resection, or segmentectomy as appropriate. We performed segmentectomy when the nodule was located deep in the middle of a segment

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or could not be located intraoperatively. A frozen-section specimen was examined intraoperatively to identify Noguchi type A or B lesions, as described in detail in our previous report.[1] If the tumor was confirmed as Noguchi type A or B with a resection margin greater than 1 cm, the wound was closed and the patient followed up on an outpatient basis. If the resected margin was insufficient, an additional margin was resected. If the tumor was a primary malignancy, but not Noguchi type A or B lesions, lobectomy and systematic lymph node dissection were additionally performed. Patients were followed up on an outpatient basis at a minimum of every 6 months by physical check-up, plain chest radiography, and laboratory testing. Patients who underwent limited resection for Noguchi type A or B lesions had chest CT scans taken every year.

Of the 36 patients who were given intraoperative diagnoses of Noguchi type A or B tumors or nonmalignancy, 30 underwent wedge resection and six underwent segmentectomy. We performed lobectomy and lymph node dissection in 14 patients with intraoperative diagnoses of type C tumors. The final pathologic diagnosis of 50 cases was 40 adenocarcinomas, including two Noguchi type A, 23 type B, and 15 type C tumors, five atypical adenomatous hyperplasias, four fibroses, and one granuloma.[1]

Statistical Analysis

The primary endpoint for analyses was the recurrence-free proportion of patients, measured from the date of surgery to the date when possible recurrence was suspected for the first time. The last follow-up observation was censored when the patient was alive without any signs of recurrence, was lost to follow-up, or died from any cause other than lung cancer. We also measured overall survival from the date of surgery to the date of death from any cause. The recurrence-free proportion curve was plotted using the Kaplan–Meier method and the statistical significances of difference between the subgroups were determined by the two-sided log-rank test. A p value of less than 0.05 was considered to represent a statistically significant difference. We used statistical analysis software, Dr. SPSS II for Windows, Standard Version 11.0 (SPSS Inc., Chicago, IL) for all analyses.

RESULTS

Long-Term Outcome Among 40 Adenocarcinoma Patients

After the preliminary results were published, we continued to observe the 40 adenocarcinoma patients with a median follow-up period of 10 years. There were no patients with recurrence within the first 5 years. However, in four patients who had undergone limited resection, pulmonary adenocarcinoma developed in the area surrounding the cut-end staples of the initial limited resection 5 years or later after the initial surgery. Of these, three had Noguchi type B tumors, and one had a type C tumor, which was initially diagnosed as type B by frozen-section examination. Therefore, this patient only underwent wedge resection. After detailed discussion, he decided not to have any further treatment on his own will. All the other 14 type C patients who underwent lobectomy and lymph node dissection are free from recurrence or metachronous primary disease at the time of writing (Fig. 1).

The characteristics of the four patients in whom pulmonary adenocarcinoma developed around the area of the cut-end scar are shown in the Table 1. All of them initially underwent wedge resection, and had intraoperative frozen-section diagnoses of Noguchi type B. We reported the findings in patients No. 1 to 3 in detail previously.[3] Solid nodular shadows appeared at the cut-ends of initial limited-resection sites, more than 5 years after the initial surgery in all four patients. These secondary lesions were cytohistologically diagnosed as adenocarcinomas, and they showed the same epidermal growth factor receptor mutation status as the primary lesions in three of the four patients. Therefore, we diagnosed them to be possible cut-end recurrences rather than metachronous primary diseases.

Figure 2*A* shows the recurrence-free proportion curve of the 40 adenocarcinoma patients. The 5- and 10-year recurrence-free proportions were 100% and 92%, respectively. There was one patient who died from lung cancer and one who died from other malignant disease. The overall 5- and 10-year survival rates were 100% and 95%, respectively. Figure 2*B* shows the recurrence-free proportion curve of the 26 patients who underwent limited resection, in comparison with that of the 14 patients who underwent lobectomy. The 5- and 10-year recurrence-free proportions of the limited-resection patients were 100% and 87%, respectively. There were no statistically

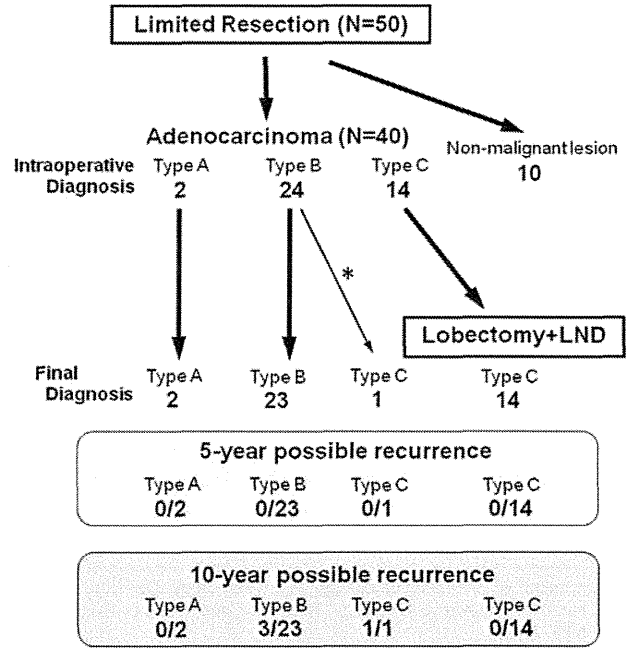

FIGURE 1. The intraoperative and final pathologic diagnosis, operative procedure, and long-term outcome of 40 adenocarcinoma patients; 1 patient had a type C tumor which was intraoperatively diagnosed as type B by frozen-section examination, and this patient underwent wedge resection only (asterisk). LND, lymph node dissection.

TABLE 1. Characteristics of Four Patients with Possible Recurrence

No.	Size (mm)	Final Diagnosis	Site of Initial Recurrence	Time to Recurrence (Yrs)	Status of EGFR Mutation Primary / Recurrence	Treatment for Recurrence	Outcome (Yrs)
1.	20	Type B	Cut-end scar	7.9	L858R and S768I/L858R	Reoperation	Alive (11.6)
2.	11	Type B	Cut-end scar	5.5	E746-A750 del type 2/E746-A750 del type 2	Reoperation	Alive (9.8)
3.	20	Type C	Cut-end scar and pleural effusion	8.0	None/none	EGFR-TKI	Dead (9.0)
4.	15	Type B	Cut-end scar and contralateral lung	10.5	L858R/L858R	EGFR-TKI	Alive (12.1)

EFGR, epidermal growth factor receptor. TKI, tyrosine-kinase inhibitor.

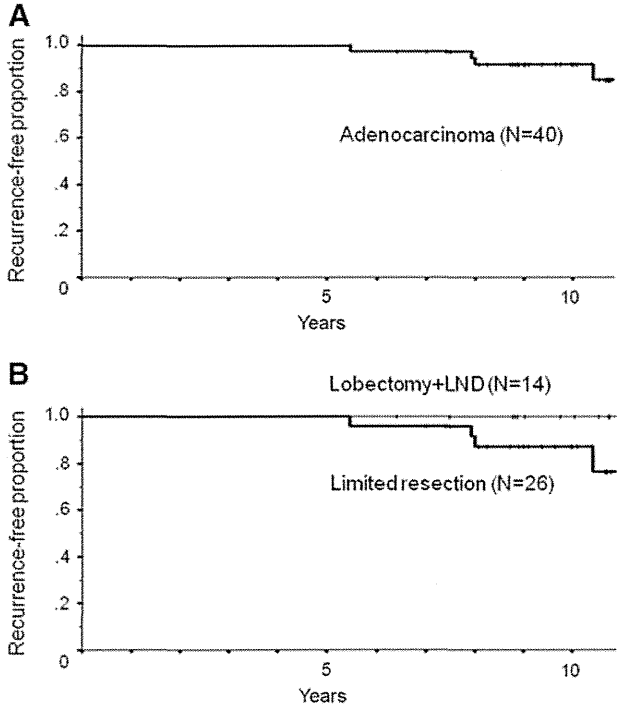

FIGURE 2. ***A***, The recurrence-free proportion curves of 40 adenocarcinoma patients, and (***B***) 26 patients who underwent limited resection, in comparison with 14 patients who underwent lobectomy. LND, lymph node dissection.

significant differences in recurrence-free proportion between the limited resection and lobectomy groups ($p = 0.136$).

DISCUSSION

During the monitoring of the patients who had undergone limited resection (median follow-up period, 10 years), three patients with Noguchi type B and one with Noguchi type C tumors at the final pathologic diagnosis developed pulmonary adenocarcinoma 5 years or more after limited resection. In all cases, the second cancers developed in the areas surrounding the initial limited-resection cut-end staples, which strongly suggests they were cut-end recurrences rather than metachronous primary cancer. However, in one of the four patients, the second cancer harbored only one of the two point mutations observed in the *EGFR* gene of the first cancer, and therefore we strongly speculate that this second cancer was a metachronous primary lesion.[3] Regardless of the etiology, the disease-control rate of the limited resection in our trial was unacceptably low, at 85% (22 of 26). The minimum resection margin of greater than 1 cm in our trial setting may have been insufficient to completely remove cancer cells by limited resection.

Surgical resection was not indicated in two of the four patients because their second cancer in these areas had already disseminated into the thoracic cavity upon detection. Although there were no statistically significant differences in recurrence-free proportion between the limited resection and lobectomy groups in the current series, this lack of significance have been caused by a beta error. All 14 patients with invasive type C adenocarcinoma who underwent standard lobectomy and lymph node dissection remain free from recurrence or metachronous cancer at the time of writing this article. Lobectomy might be the curative treatment of choice even for small GGO adenocarcinomas. Care must be taken in determining the indication for limited resection, which should be assessed further in a trial setting.

The most notable result in the present study is the length of time to recurrence. In our first report, we suggested that a 5-year follow-up period is insufficient to conclude that the disease is cured, considering the probable slow-growing nature of GGO lesions, and that an additional 5 years of monitoring is necessary to yield definitive conclusions.[1] We previously described the considerable risk of the late recurrence of non–small-cell lung cancer more than 5 years after curative surgery.[5,6] These findings prompted us to continue to follow-up of limited-resection patients in whom we observed possible cut-end recurrence 5 years or more after limited resection. There are several prospective studies of limited resection for pulmonary adenocarcinoma reported from Japan.[7–10] In these studies, wedge resection was performed mostly for intraoperatively diagnosed noninvasive adenocarcinomas, and no recurrences or cancer-related deaths were observed. However, these results were obtained after a relatively short median follow-up period of 29 to 51 months, which may have been insufficient to determine these slow-growing cancers as cured.

In Japan, a single-arm, limited-resection trial, Japanese Clinical Oncology Group (JCOG) 0804/West Japan Oncology Group (WJOG) 4507L is ongoing for subsolid T1aN0M0[11] peripheral tumors with a consolidation–tumor size ratio of 0.25 or less. A total of 330 patients had been enrolled as of April 2011, and therefore the 10-year follow-up results will not be available until 2020. Another two major randomized, limited-resection trials, JCOG0802 / WJOG4607L and Cancer

and Leukemia Group B-140503, are still in the accumulation phase in Japan and in the United States and Canada, respectively. Their aims are to compare limited resection with standard lobectomy for T1aN0M0 peripheral non–small-cell lung cancer. These are ambitious projects that could raise the standard of lung cancer surgery. However, considering the possibility of delayed cut-end recurrence, clinicians should refrain from indicating limited resection in clinical practice until the abovementioned trials clearly show that limited resection is the treatment of choice for these types of small lung cancer. Currently at our department, for a subsolid T1aN0M0 peripheral tumor with a consolidation–tumor size ratio of 0.25 or less, we indicate limited resection only when the patient's consent is obtained after discussion based on our in-house and JCOG0804/ WJOG4507L limited-resection experiences. For a tumor with a consolidation–tumor size ratio of greater than 0.25, we ask the patient to participate in JCOG0802 / WJOG4607L trial. If the patient agrees to participate, he or she is randomly allocated to segmentectomy or lobectomy. If not, we indicate standard lobectomy and node dissection.

CONCLUSIONS

To the best of our knowledge, this is the first report in the literature on the long-term outcome of a prospective limited-resection trial of pulmonary small GGO lesions after a median follow-up period of 10 years. The findings of four possible cases of delayed cut-end recurrence clearly demonstrate the need for long-term follow-up of more than 5 years, and that limited resection should still be done only in a trial setting, even for small GGO cancer.

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ORIGINAL ARTICLE – THORACIC ONCOLOGY

Characteristic Immunophenotype of Solid Subtype Component in Lung Adenocarcinoma

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ABSTRACT

Background. Lung adenocarcinomas represent a morphologically heterogeneous tumor composed of an admixture of different histologic subtypes (lepidic, papillary, acinar, and solid subtype). The presence of a solid subtype component is reported to be associated with a poorer prognosis. The aim of this study was to evaluate the characteristic immunophenotype of the solid subtype component compared with the immunophenotypes of other components.

Methods. We analyzed the clinicopathological characteristics of stage I adenocarcinoma patients with predominant solid subtype disease. Furthermore, we immunostained adenocarcinomas with predominant lepidic, papillary, acinar, and solid subtype components ($n = 23$ each) for 10 molecular markers of tumor invasiveness and scored the results.

Results. Patients showing predominance of the solid subtype component (solid subtype adenocarcinoma) had a poorer prognosis than those showing predominance of the lepidic, papillary, or acinar component. Lymphovascular invasion was more often detected in solid subtype tumors than in others. The solid subtype component showed a significantly stronger staining intensity of laminin-5 expression than the lepidic, papillary, and acinar components ($P < 0.001$, $P < 0.001$, and $P = 0.016$, respectively). The fibronectin and vimentin expression levels were also significantly higher in the solid subtype component than in other components. This immunostaining character was validated by using mixed-subtype adenocarcinomas containing all four components in the same tumor.

Conclusions. This study concluded that the solid subtype component in lung adenocarcinomas exhibit the invasive immunophenotype, including increased laminin-5 expression, compared with the other components, which may be associated with a poorer prognosis.

Lung cancer is the leading cause of cancer-related deaths in Japan.[1] Lung adenocarcinoma is the most frequently encountered histologic type of lung cancer, and the incidence rate of adenocarcinoma is increasing in Japan.[1] Lung adenocarcinomas are known to have heterogeneous morphologic features and to have diverse biological properties. The 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society International Multidisciplinary Classification Panel classified invasive adenocarcinoma according to their predominant subtype using a method called comprehensive histologic subtyping to semiquantitatively estimate the percentages of the subtypes present within the tumors: lepidic, papillary, acinar, micropapillary, and solid patterns.[2–4] The lepidic subtype is introduced as neoplastic cell replacement with retained normal alveolar architecture. The papillary subtype shows fingerlike tumor cell projections with a stromal core; the acinar subtype demonstrates the characteristic glandular pattern; and the solid subtype demonstrates a more compressed structure without the features associated with the other main subtypes. Micropapillary adenocarcinoma is introduced as a histologic subtype, and if it is the predominant pattern, it is classified as micropapillary predominant adenocarcinoma. Though subtyping system has been widely

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adopted, most adenocarcinomas (more than 80 %) are classified into the mixed subtype, representing a combination of the different histologic subtypes; thus, most adenocarcinomas usually consist of highly heterogeneous components.[5]

It is becoming increasingly clear that the presence of specific histologic patterns likely drives the biologic behavior of the tumor.[6] Several studies have shown that lepidic-predominant adenocarcinoma exhibit relatively quiet behavior and are associated with a better prognosis.[7–10] On the other hand, a recent study reported that the predominance of the solid component shows unfavorable outcomes, suggesting that these tumors may be more invasive and more likely to metastasize than the subtypes of tumors without a solid component.[11–14]

Although some molecular markers of more aggressive behavior of lung adenocarcinomas have been identified (including laminin-5, E-cadherin, vimentin, and SOX-2), the molecular mechanisms underlying the aggressive behavior of lung adenocarcinomas with a solid component are not yet well understood.[15–17] Therefore, it is important to investigate the biological characteristics of each histologic subtype of adenocarcinoma, paying particular attention to the solid subtype. However, to our knowledge, until now, no studies have reported the biologic characteristics of the solid subtype of adenocarcinoma as distinct from those of the other subtypes.

In this background, this study was conducted to investigate the biologic characteristics of the solid subtype of adenocarcinomas in comparison with those of the other histologic subtypes.

MATERIALS AND METHODS

Subjects

Of 1256 consecutive patients who underwent surgical resection for primary lung cancer between January 2003 and December 2007 at the National Cancer Center Hospital East, Chiba, Japan, histologic slides of primary pulmonary lung adenocarcinoma, pathological stage I, from 638 patients were enrolled. We reviewed patient clinicopathologic information, including histologic subtype (lepidic, papillary, acinar, micropapillary, and solid), from the medical records, as well as the prognosis of each patient.

All specimens were collected after obtaining informed consent from the patients, and the study was conducted with the approval of the Institutional Review Board of the National Cancer Center.

Histologic Evaluation

The surgical specimens were fixed with 10 % formalin and embedded in paraffin. Serial 4-μm sections were stained by the H&E method, by the Alcian blue–periodic acid–Schiff staining method to detect cytoplasmic mucin production, and by the Victoria blue–van Gieson staining method for elastic fibers. The sections were reviewed by two pulmonary pathologists (T.T. and G.I.) who were unaware of the clinical data. Invasive lung adenocarcinomas were classified according to the 2011 International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinomas into the following subtypes: lepidic predominant, papillary predominant, acinar predominant, micropapillary predominant, and solid predominant pattern.[2–4] Histologic subtyping was performed in the primary tumor in a semiquantitative manner, with the percentage of the five possible histologic subtypes quantified in 10 % increments, totaling 100 % per tumor, as described earlier.[18] The predominant component was defined as the histologic component that comprised the largest percentage among the components.

Pathological stage was determined on the basis of the tumor, node, metastasis staging classification of the International Union Against Cancer.[19]

Tissue Microarray Construction

From the consecutive surgical samples obtained from January 2009 to December 2010, we selected 23 cases of adenocarcinoma with predominance of the solid component (solid component accounting for over 50 % of all components; solid-predominant adenocarcinoma). We also selected 23 cases of adenocarcinoma with predominance (accounting for greater than 50 % of all components) of the lepidic component (lepidic-predominant adenocarcinoma), papillary component (papillary-predominant adenocarcinoma), and acinar component (acinar-predominant adenocarcinoma). No micropapillary-predominant adenocarcinoma was detected in our specimens. The most representative tumor lesions, showing the predominant histologic subtype of the tumors, were carefully selected and marked on hematoxylin and eosin–stained slides for the construction of tissue microarrays (TMAs). TMA was constructed with a manual tissue-arraying instrument (Azumaya, Tokyo, Japan), as previously described.[20,21] Specimens were routinely sampled by taking a core sample of each tumor from two different areas. Specimens from the 23 cases for each subtype were punched, and a total of 92 cases of core samples were mounted on the recipient blocks. A normal control TMA that included samples from nonmalignant specimens of lung tissue was used as a positive control.

Immunohistochemical Staining

The 10 molecular markers selected for investigation in this study are listed in Table 1. The antibodies consisted of markers for cellular adhesion molecules and epithelial–mesenchymal transition (EMT) markers. The immunohistochemical staining procedures were as follows. TMA recipient blocks were cut into 4-μm sections and mounted on silane-coated slides. The sections were deparaffinized in xylene and dehydrated in a graded ethanol series. After the sections were washed with distilled water, they were placed in 0.1 M of citric acid buffer. Antigen retrieval of E-cadherin, N-cadherin, intracellular adhesion molecule 1 (ICAM-1), CD44, fibronectin, vimentin, and matrix metalloproteinase (MMP) 7 and MMP9 was performed by placing the slides in a microwave oven (H2800 Microwave Processor; Energy Beam Sciences, East Granby, CT) and heating them at 95 °C for 20 min. Antigen retrieval for ZEB-1 was performed by placing the slides in an autoclave oven (Dako Pascal; DakoCytomation, Carpinteria, CA) and heating them at 121 °C for 4 min and 90 °C for 45 min, and that for laminin-5 was performed with proteinase K (ready to use, code S3020, Dako North America, Carpinteria, CA). The process of antigen retrieval is shown in detail in Table 1. The slides were washed three times in phosphate-buffered saline (PBS) and immersed in a 0.3 % hydrogen peroxide solution in methanol for 15 min to inhibit endogenous peroxidase activity. They were again washed three times in PBS, and nonspecific binding was blocked by preincubation with 2 % normal swine serum in PBS (blocking buffer) for 30 min at room temperature. Individual slides were then incubated with primary antibodies at a final dilution in the blocking buffer. The slides were washed again and incubated with EnVision (Dako, Glostrup, Denmark) for 1 h at room temperature. Color reaction was developed in 2 % 3,30-diaminobenzidine in 50 mM Tris buffer (pH 7.6) containing 0.3 % hydrogen peroxidase. Finally, the slides were counterstained with Meyer hematoxylin.

Calculation of Immunohistochemical Scores

All the stained tissue sections were semiquantitatively scored and evaluated independently under a light microscope by two pathologists (T.T. and G.I.); when the evaluation results differed, the final report was provided on the basis of a consensus reached between the two pathologists by evaluation of the slides together under a conference microscope. The immunostaining score was evaluated on the basis of the staining intensity and the percentage of cells that showed positive staining. The following scoring system was used: 0 (negative staining, defined as no immunoreactivity); 1 + (weak staining intensity); 2 + (strong staining intensity). We also evaluated the extent of staining in a lesion as a percentage (0–100 %). The staining scores were calculated by multiplying the percentage values by the staining intensity, with the scores ranging from 0 to 200. The average of the staining scores obtained for two core samples from the same specimen was determined, and the result was recorded as the score for that case. In case of the loss of tumor cells in one of the two samples, the staining score was calculated from the data for the remaining sample.

Validation Study Using Full Cross Sections of Adenocarcinoma

In the TMA examination, we found significantly higher expression levels of laminin-5, fibronectin, and vimentin in the solid subtype than in the nonsolid subtypes of adenocarcinoma. We validated the expression levels of these molecules in each subtype using full cross sections of the

TABLE 1 Antibodies used

Antibody	Clone	Preparation	Dilution	Source
E-cadherin	NCH-38	Microwave	1:50	DakoCytomation, Carpinteria, CA
N-cadherin	6G11	Microwave	1:50	DakoCytomation, Carpinteria, CA
ICAM-1	sc-8439	Microwave	1:50	Santa Cruz, Santa Cruz, CA
CD44	DF1485	Microwave	1:50	Novocastra, Newcastle, UK
Laminin5	D4B5	ProteinaseK	1:200	Chemicon, Temecula, CA
Fibronectin	568	Microwave	1:100	Leica Biosystems, Newcastle, UK
Vimentin	V9	Microwave	1:50	DakoCytomation, Carpinteria, CA
ZEB-1	HPA027524	Autoclave	1:250	Sigma-Aldrich, St. Louis, MO
MMP7	141–7B2	Microwave	1:50	Daiichi Fine Chemical, Toyama, JP
MMP9	15W2	Microwave	1:50	Leica Biosystems, Newcastle, UK

ICAM-1 intracellular adhesion molecule 1, *MMP* matrix metalloproteinase

adenocarcinomas containing all the four histologic components in the same tumor. We selected 20 adenocarcinomas containing all the four histologic subtypes blocked in the same slide. We evaluated the immunohistochemical scores for the histologic subtypes by staining these full cross sections. Two most representative lesions showing each of the histologic components (lepidic, papillary, acinar, and solid) were carefully selected and marked on hematoxylin-eosin-stained slides and the immunohistochemical scores were evaluated in the same way, as described earlier. The average staining score for two lesions from the same specimen was determined, and the result was recorded as the score for that case.

Statistical Analysis

The Pearson Chi-square test was used to determine the statistical significance of the differences between two groups. For the univariate analysis, cumulative survival was estimated by the Kaplan-Meier method, and differences in variables were calculated by the log-rank test. The end point for the analyses was overall survival measured from the date of surgery to the date of death from any cause, or the date until which the patient was last known to be alive. The Mann-Whitney *U*-test was used to compare the staining scores. All of the reported *p* values were two sided, and the significance level was set at less than 0.05. The analyses were performed with the SPSS 11.0 statistical software program (Dr. SPSS II for Windows, standard version 11.0; SPSS, Chicago, IL).

TABLE 2 Clinicopathologic factors

Characteristic[a]	No. of cases	Histologic subtype of predominant component			
		Solid	Lepidic	Papillary	Acinar
All cases	638	86	237	244	71
Age					
<65 y	327	44	124	127	32
>65 y	311	42	113	117	39
Gender					
Male	314	65	109	109	31
Female	324	21	128	135	40
p			<0.001	<0.001	<0.001
Smoking status					
Smoker	336	69	111	120	36
Nonsmoker	297	15	125	122	35
p			<0.001	<0.001	<0.001
Tumor size					
>3 cm	471	50	191	180	50
<3 cm	167	36	46	64	21
p			<0.001	0.007	
Lymphovascular invasion					
Positive	164	52	19	64	29
Negative	474	34	218	180	42
p			<0.001	<0.001	0.015
Pleural invasion					
Positive	112	32	12	44	24
Negative	498	52	210	192	44
p			<0.001	<0.001	

[a] *p* value is for solid predominant tumor vs. nonsolid predominant tumor (lepidic, papillary, acinar)

RESULTS

Patient Characteristics

The clinicopathologic characteristics of the 638 pathological stage I adenocarcinoma patients are shown in Table 2. The median age of the patients was 65 years (range 20–92 years), and the male to female ratio was 50.7 %. Of the total, 53.5 % of the patients were either current or former smokers. The median size of the tumors was 2.3 cm (range 0.5–22.2 cm). Of the 638 patients, 69 underwent sublobar resections (wedge resections or segmentectomies), 566 patients underwent lobectomy, and the remaining three patients underwent pneumonectomy. The median follow-up time was 54.8 months. The majority of the tumors were composed predominantly of the lepidic (237 cases; 37.1 %) or papillary (244 cases; 38.2 %) components, followed in frequency by the solid (86 cases; 13.5 %) and acinar (71 cases; 11.1 %) components. No micropapillary predominant adenocarcinoma was detected in our samples. Adenocarcinoma with fetal components was detected in one case, which was excluded from the analysis.

Predominant Histologic Subtype and Clinical Outcome

The overall survival curves constructed by the Kaplan-Meier method are shown according to the predominant histologic subtype in Fig. 1. The estimated 5-year survival rate in patients with solid subtype adenocarcinoma was 74.2 %, as opposed to that in those with the lepidic, papillary, or acinar subtype of adenocarcinoma, in whom it was 92.9 %, 84.5 %, and 82.1 %, respectively. Patients with the solid subtype of adenocarcinoma showed a significantly poorer prognosis than those with the lepidic and papillary subtypes of the tumor ($P = 0.001$ % and 0.012 %, respectively). Patients with the solid subtype of adenocarcinoma also showed a poorer prognosis than those with the acinar subtype, although this difference was not statistically significant ($P = 0.076$).

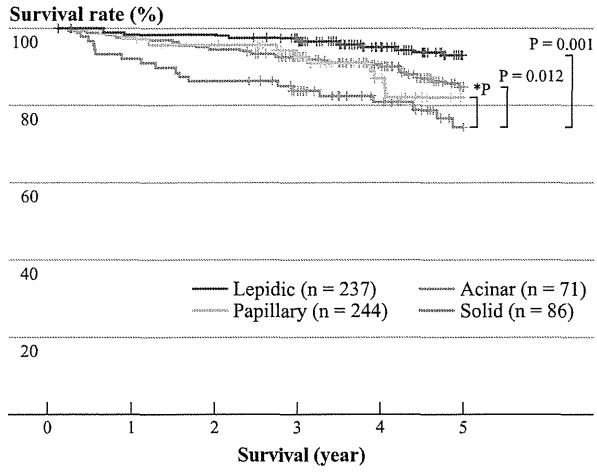

FIG. 1 Relation between the subtype of adenocarcinoma classified according to the predominant cellular component in the tumor and the patient survival. Overall survival curves after surgery grouped by the predominant histologic component in the tumor, calculated by the Kaplan-Meier method. Patients with the solid subtype of adenocarcinoma showed significantly poorer survival than those with the lepidic or papillary subtype of tumors (log-rank test, $P = 0.001$ and $P = 0.012$, respectively). The trend was consistent between patients with the solid and acinar subtypes of adenocarcinoma, although it was only of marginal statistical significance ($*P = 0.076$)

Predominant Histologic Subtype and Pathologic Characteristics

The correlations between the predominant histologic component (tumor subtype), focusing on the solid component, and the pathologic features were as follows. Lymphovascular invasion was observed significantly more frequently in the solid subtype of adenocarcinoma than the lepidic, papillary, or acinar subtype of the tumor ($P = 0.001$, $P = 0.001$, $P = 0.015$, respectively). Pleural invasion and larger tumor size was encountered more frequently in the solid subtype than in the lepidic or papillary subtype of adenocarcinoma (Table 2).

Immunohistochemical Scores in the Solid Subtype Adenocarcinomas

Previous studies have suggested that predominance of the solid component in a lung adenocarcinoma might be indicative of the aggressive nature of the tumor. Ten antibodies associated with cancer cell invasiveness were selected for this investigation. Immunohistochemical analysis was performed focusing on comparison between the solid and other subtypes of lung adenocarcinoma.

The median staining score ± standard deviation (SD) for laminin-5 in the solid subtype adenocarcinomas was 70.0 ± 47.1, which was significantly higher than that in the lepidic (7.5 ± 16.0), papillary (10.0 ± 30.6), and acinar (22.5 ± 24.0) subtypes of the tumor (Fig. 2a–d). The staining score for fibronectin in the solid subtype adenocarcinomas was 12.5 ± 36.3, which was significantly higher than that in the lepidic (0.0 ± 1.9), papillary (2.5 ± 23.4), and acinar (0 ± 33.9) subtypes of the tumor (Fig. 2e–h).

The median staining score ± SD for vimentin in the solid subtype adenocarcinomas was 2.5 ± 28.2, which was significantly higher than that in the lepidic (0.0 ± 0.0), papillary (0.0 ± 9.16), and acinar (0.0 ± 8.91) subtypes of the tumor (Fig. 2i–l). The expression levels of laminin-5, fibronectin, and vimentin were significantly higher in the solid subtype adenocarcinomas than in the other subtypes (lepidic, papillary, and acinar) of the tumor (Fig. 3e–g).

The median staining score ± SD for E-cadherin in the solid subtype adenocarcinomas was 62.5 ± 48.6, and the scores in the lepidic, papillary, and acinar subtypes of the tumor were 32.5 ± 24.3, 35.0 ± 42.5, and 25.0 ± 36.5, respectively. The staining score for ICAM-1 in the solid subtype tumors was 82.5 ± 32.7, and the scores in the lepidic, papillary, and acinar subtypes of the tumor were 85.0 ± 45.6, 40.0 ± 48.3, and 45.0 ± 41.3, respectively. The expression levels of E-cadherin and ICAM-1 in the solid subtype adenocarcinomas were significantly higher than those in the acinar subtype of the tumor (Fig. 3a, c). The staining score for CD44 in the solid subtype adenocarcinomas was 95.0 ± 59.9, and the scores in the lepidic, papillary, and acinar subtypes of the tumor were 57.5 ± 42.4, 15.0 ± 54.2, and 12.5 ± 38.6, respectively. The CD44 expression level in the solid subtype adenocarcinomas was significantly higher than the levels in the papillary and acinar subtypes of the tumor (Fig. 3d). The staining scores for N-cadherin in the lepidic, papillary, acinar, and solid subtypes of adenocarcinoma were 0.0 ± 0.0, 0.0 ± 0.0, 0.0 ± 5.764, and 0.0 ± 8.37, respectively. The staining scores for ZEB-1 in the lepidic, papillary, acinar, and solid subtypes of adenocarcinoma were 0.0 ± 0.0, 0.0 ± 0.0, 0.0 ± 0.0, and 0.0 ± 31.2, respectively. The staining scores for MMP7 in the lepidic, papillary, acinar, and solid subtypes of adenocarcinoma were 7.5 ± 6.8, 17.5 ± 26.1, 15.0 ± 33.3, and 17.5 ± 24.9, respectively. The staining score for MMP9 in lepidic, papillary, acinar, and solid subtypes was 62.5 ± 28.2, 87.5 ± 32.7, 55.0 ± 32.5, and 68.7 ± 32.7, respectively. There were no significant differences between the four tumor subtypes in the expression levels of N-cadherin, ZEB-1, MMP7, or MMP9 (Fig. 3b, h–j).

Immunohistochemical Analysis of the Solid Subtype of Adenocarcinomas: Validation Study Using Full Cross Sections of Adenocarcinoma

In the TMA examination, we found significantly higher expression levels of laminin-5, fibronectin, and vimentin in the solid subtype than in the other subtypes of

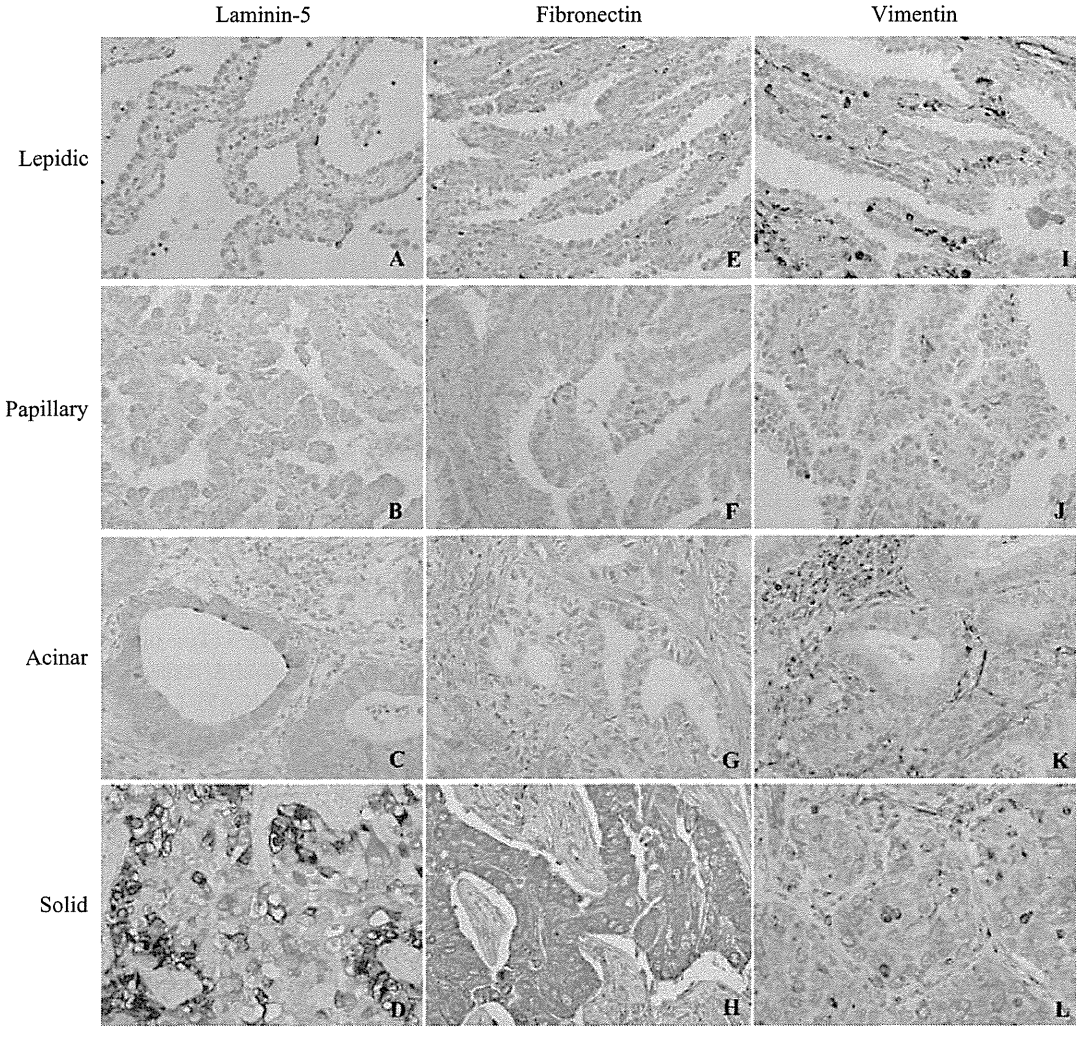

FIG. 2 Immunohistochemical findings according to the histologic subtype of lung adenocarcinoma using the TMA method. **a–d** Immunohistochemical staining for laminin-5. Staining for laminin-5 expression was stronger and more frequent in the solid subtype (**d**) than in the lepidic (**a**), papillary (**b**), or acinar (**c**) subtypes of adenocarcinoma. **e–h** Immunohistochemical staining for fibronectin. Positive staining was more frequently detected in the solid subtype (**h**) than in the lepidic (**e**), papillary (**f**), or acinar (**g**) subtypes of adenocarcinoma. **i–l** Immunohistochemical staining for vimentin. A few cases of the solid subtype **l** showed positive expression; however, all cases of the lepidic (**i**), papillary (**j**), and acinar (**k**) subtypes were negative

adenocarcinoma. We validated the expression levels of these molecules in each subtype using full cross sections of 20 adenocarcinomas containing all four histologic components within the same tumor. The median staining score ± SD for laminin-5 in the solid component was 70.0 ± 50.0, and the scores in the lepidic, papillary, and acinar components were 10.0 ± 18.1, 10.0 ± 26.4, and 30.0 ± 34.1, respectively. The staining score for fibronectin in the solid component was 15.0 ± 28.7, and the scores in the lepidic, papillary, and acinar components were 0.0 ± 3.9, 0.0 ± 18.4, and 0.0 ± 12.8, respectively. The staining score for vimentin in the solid component was 5.0 ± 12.9, and the scores in the lepidic, papillary, and acinar components were 0.0 ± 5.2, 0.0 ± 6.9, and 0.0 ± 12.7, respectively. Thus, the expression levels of laminin-5, fibronectin, and vimentin in the solid lesions were significantly higher than those in the other lesions (Figs. 4, 5).

DISCUSSION

In the present study, we analyzed the correlation between the predominant histologic component in lung adenocarcinoma and the clinical outcome and clinicopathologic characteristics in surgically resected cases of stage I lung adenocarcinoma. Consistent with previous

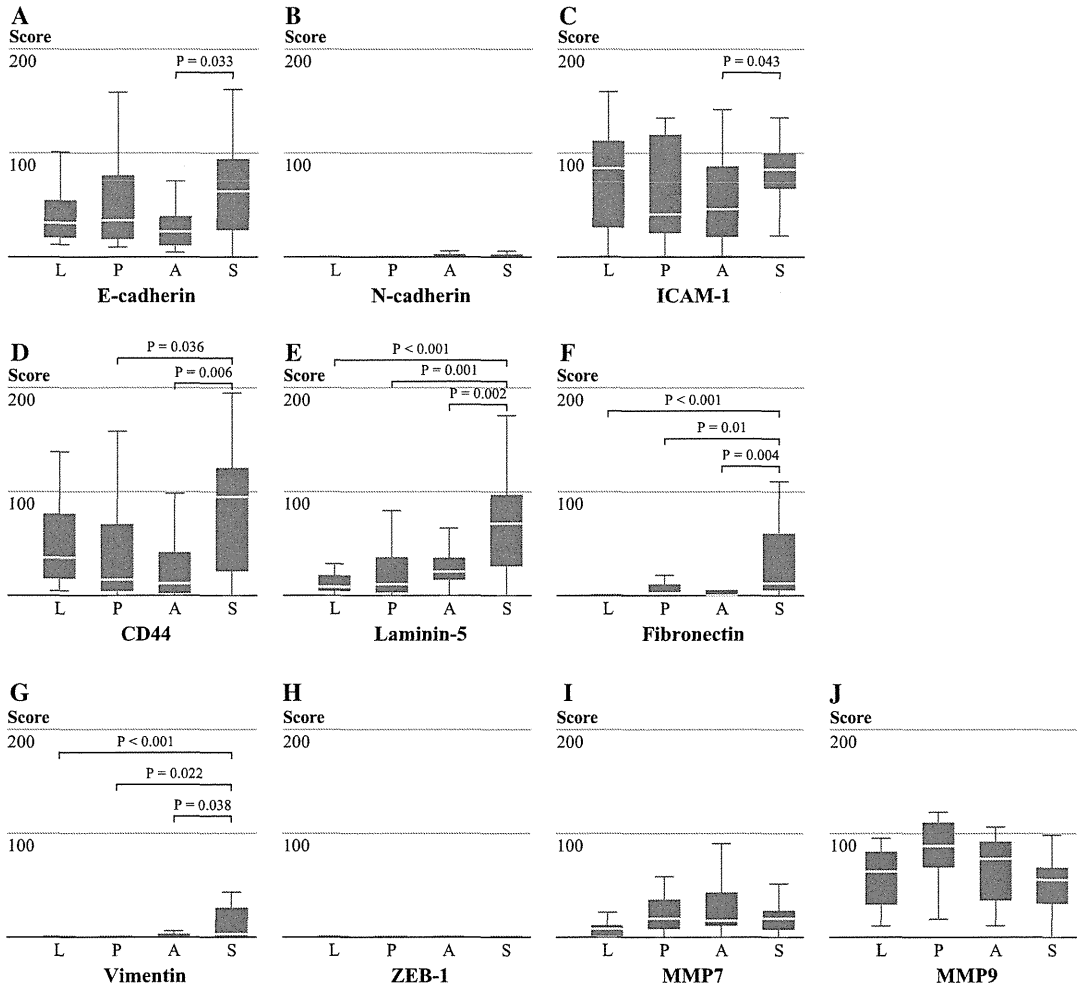

FIG. 3 Immunohistochemical scores according to the histologic subtype sampled from the predominant subtype. **a** E-cadherin. **b** N-cadherin. **c** Laminin-5. **d** CD44. **e** ICAM-1. **f** Fibronectin. **g** Vimentin. **h** ZEB-1. **i** MMP7. **j** MMP9. *L* lepidic. *P* papillary. *A* acinar, *S* solid

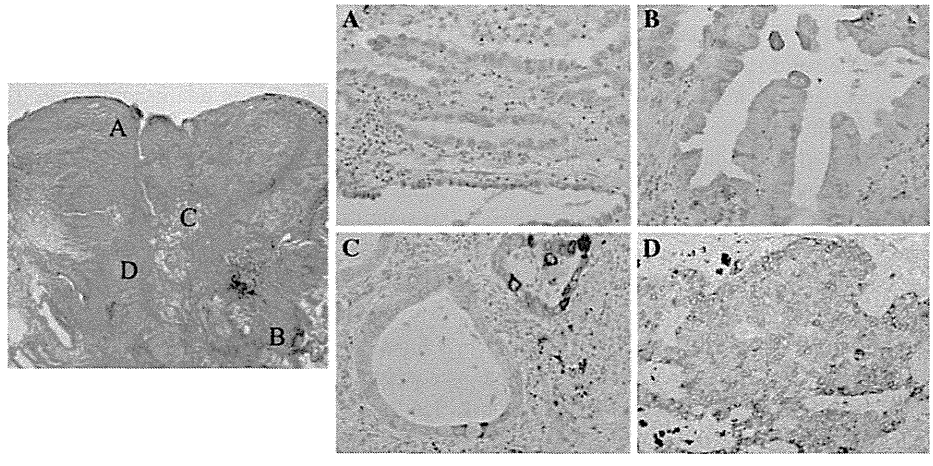

FIG. 4 Immunohistochemical staining for laminin-5 in full cross sections of adenocarcinomas, which contain all the four histologic components within the same tumor. **a** Lepidic. **b** Papillary. **c** Acinar. **d** Solid. The immunohistochemical expression of laminin-5 was more frequent in the solid component than in the lepidic, papillary, or acinar components

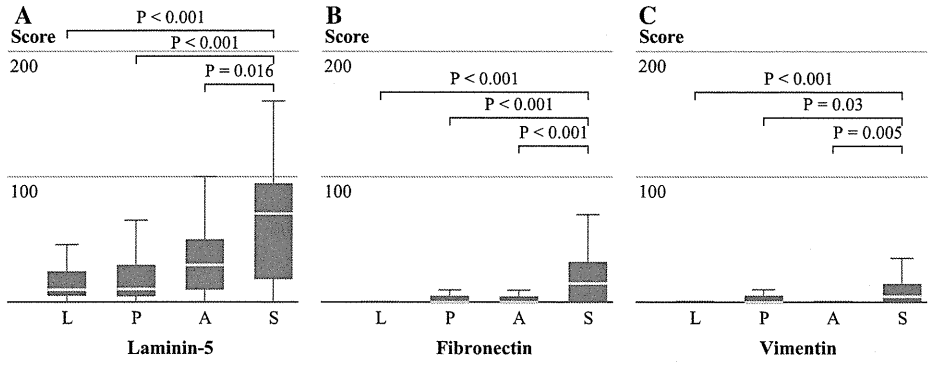

FIG. 5 Comparison of the immunohistochemical scores in full cross sections of adenocarcinomas, which contain all the four histologic components (lepidic, papillary, acinar, and solid) within the same tumor. **a** Laminin-5. **b** Fibronectin. **c** Vimentin. The score for laminin-5 expression was significantly higher in the solid component than in any of the other (lepidic, papillary, and solid) components. The staining scores for fibronectin and vimentin expression were also significantly higher in the solid component than in any of the other (lepidic, papillary, and acinar) components

reports, patients with solid subtype adenocarcinomas had a significantly poorer prognosis than those with the other subtypes of adenocarcinoma. Lymphovascular invasion was significantly more frequent in the solid subtype tumors.[12,14,18,22] These clinicopathologic findings suggest that the predominance of the solid component may reflect a biologically more aggressive nature of the tumor and also a more invasive nature of the tumors than that of other nonsolid components.

The reason why predominance of the solid component in lung adenocarcinoma was associated with a significantly poorer prognosis could not be clarified. Although some researchers have reported markers of aggressive tumor behavior in lung adenocarcinomas using immunohistochemical staining, there has been no detailed assessment of the molecular features of each histologic component of adenocarcinomas. The immunohistochemical profile of the solid component in lung adenocarcinomas had not been well understood.[17,23–26]

In the current study, the most remarkable immunohistochemical finding of the solid subtype of adenocarcinoma was the increased expression of laminin-5. The expression of laminin-5 was significantly higher in the solid subtype of adenocarcinomas compared with that in the lepidic, papillary, and acinar subtypes of the tumor. Although the expressions of fibronectin and vimentin were also statistically significantly higher in the solid subtype of adenocarcinomas than in the nonsolid subtypes of the tumor, the immunoreactivity of almost all of the adenocarcinoma tissues was weak or absent.

Laminin-5 is an extracellular matrix protein that plays a key role in intercellular adhesion, cell migration, and tumor invasion. Previous work has shown that laminin-5 plays an essential role in mediating the initial adhesion of gastric carcinoma cells to the peritoneum, leading to the development of peritoneal metastasis.[27] Intensive staining of laminin-5 was found in various cancer cells that invade the stroma, including lung, gastric, colorectal, pancreatic, and oral squamous cell carcinomas.[16,28–32] These studies also reported that laminin-5 may serve as a marker of stromal invasion in lung adenocarcinoma and exhibited budding into the fibrous stroma.[16,32]

In the present study, the laminin-5 staining intensity in the solid subtype of adenocarcinoma was much higher compared with that in the nonsolid subtypes (lepidic, papillary, and acinar) of the tumor. This result indicates that strong expression of laminin-5 may be a marker of a subset of invasive adenocarcinomas, such as the solid subtype, or that laminin-5 may have a specific role in the pathogenesis of the solid subtype of lung adenocarcinoma. High expression of laminin-5 may be associated with histologic parameters, such as lymphovascular invasion and pleural invasion, which are related to stromal invasion.

Immunohistochemistry could serve as a useful tool for a more accurate histopathologic classification of adenocarcinomas, providing important information about the clinical behavior. If laminin-5 promotes progression of the solid subtype of lung adenocarcinoma, its signaling pathway could represent a new target for the treatment of lung adenocarcinomas with predominance of the solid component. Salo et al. have shown that polyclonal antibodies against the cell- and matrix-binding domains of laminin-5 target several types of carcinomas growing in vivo and effectively inhibit tumor growth and metastasis in mice.[33] More research is still needed before they can be applied for clinical use; however, antibody-mediated treatment may be potentially suitable for solid adenocarcinomas expressing laminin-5 protein.

Fibronectin regulates many cellular processes, including migration and invasion.[34,35] A previous report showed that fibronectin-induced focal adhesion kinase (FAK) activation stimulated cell migration and invasion of lung cancer, and