patients (Fig. 1a). The frequency of VI was defined as the number of invaded blood vessels on the maximum cut surface of the tumor (Fig. 1b).

Histological studies. The surgical specimens had been fixed in 10% formalin or 100% methyl alcohol at the time of surgery. The specimens were sliced through the largest diameter of the primary tumor, and all the sections were embedded in paraffin. All the serial 4-μm sections were stained using the H&E method, the Alcian blue–periodic acid–Schiff method for the detection of cytoplasmic mucin production, or the elastica van Gieson or the VVG method for the detection of elastic fibers. All the histological materials included in this series were reviewed by two pathologists (K.K. and G.I.) to ascertain the presence of VI. Intratumoral VI was defined as tumor cells existing within blood vessels with elastic fibers located inside the primary tumors (Fig. 2a,b). The pathological stage was determined based on the TNM classification of the International Union Against Cancer, 6th edition. Histological typing of the primary tumors was carried out based on the World Health Organization's classification of cell types, 6th edition. Pleural invasion was also evaluated based on whether the tumor cells had invaded the visceral pleura of the lung on elastica van Gieson- or VVG-stained sections.

Antibodies and immunohistochemical staining. E-cadherin (NCH-384; Dako Cytomation, Glostrup, Denmark), CD44 (DF1485; Novocastra, Newcastle, UK), CD44 variant 6 (VFF-7; Acris Antibodies, Herford, Germany), and vimentin (VIM 3B4; Progen Biotechnik, Heidelberg, Germany) were used as EMT-related markers. As for stromal cell markers, CD204 (SRA-E5; Trans Genic, Hyogo, Japan) was used to evaluate activated macrophages, CD34 (QBEnd 10; Dako Cytomation) was used as an endothelial cell marker, and α-SMA (1A4; Dako Cytomation) was used as a myofibroblast marker. Immunostaining was carried out using 4-μm paraffin-embedded tissue serial sections. The slides were deparaffinized in xylene and dehydrated in a graded ethanol series, and endogenous peroxidase was blocked with 3% hydrogen peroxide in absolute methyl alcohol. After epitope retrieval, the slides were washed with PBS and incubated overnight at 4°C using mouse anti-human antibodies at a final dilution of 1:100 (E-cadherin, CD44, CD44 variant 6, α-SMA, vimentin), 1:200 (CD34), or 1:400 (CD204) in the blocking buffer. The reaction products were stained with diaminobenzidine and counterstained with hematoxylin. After the confirmation of color development, the VVG method was used to examine the immunohistochemical properties of the tumor cells and the stromal cells existing in the blood vessels.

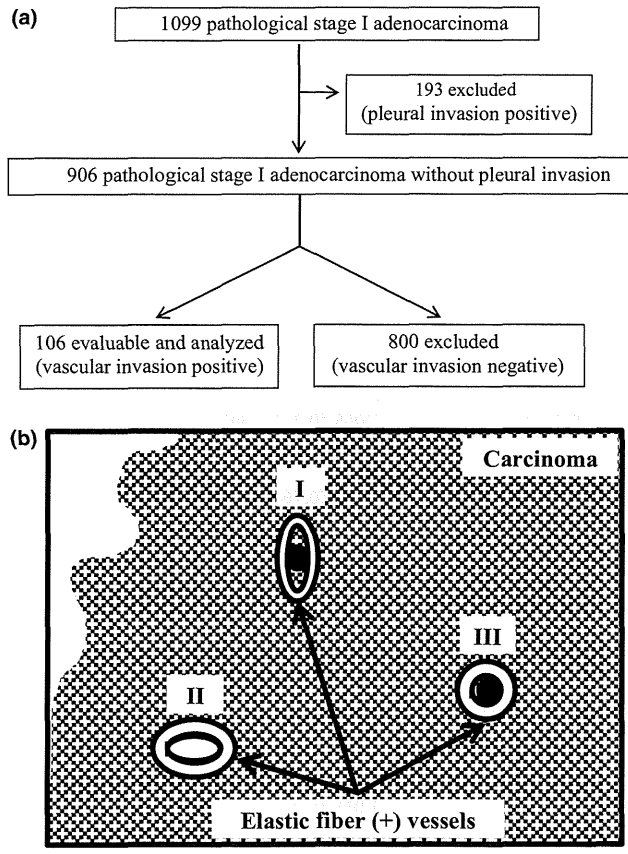

Fig. 1. (a) Scheme showing patient selection for this study. (b) Scheme showing the definition of vascular invasion. Of the three illustrated vasculatures (numbered I–III), vascular invasion is evident in two (I and III) with the presence of cancer cells (black circles).

Immunohistochemical scoring. All the stained tissue sections were semiquantitatively scored and evaluated independently under a light microscope. The labeling scores for tumor cells within intratumoral blood vessels were calculated by multiplying the percentage of positive tumor cells per lesion (0–100%) by the staining intensity level (0, negative; 1, weak; 2, strong). For CD204 and α-SMA, the numbers of positive infiltrating cells in a hot spot were counted under a high-power microscopic field (×400; 0.0125 mm^2). For CD34, the microvessel density was calculated based on the number of CD34(+) microvessels observed under a high-power microscopic field (×400; 0.0125 mm^2), based on a protocol used in a previous study.[16] We confirmed that the positive control tissues were stained by each antibody, and we also carried out negative control studies without the primary antigen for all the antibodies. When the antibody evaluations differed, the observers discussed the results, re-examined the slides, if necessary, and debated the evaluation findings until an agreement was reached. A high score was defined as a score above the median value; a low score was defined as a score below the median value.

Statistical analysis. The length of the recurrence-free period was calculated in months from the date of resection until the date of the first recurrence or last follow-up. Cumulative incidences of recurrence were estimated using the Kaplan–Meier analysis, and differences in variables were evaluated using the log–rank test. The Cox proportional hazards multivariate model was used to identify independent predictors. A backward elimination stepwise procedure was used to determine independent predictors. All the variables were initially entered into a backward stepwise analysis using P-values of 0.10 for entry and 0.05 for removal in each model. Adjusted HRs with 95% CIs were calculated for each statistically significant variable in the final stepwise model. For comparison, unadjusted HRs with 95% CIs were calculated for each candidate variable using univariate Cox analyses to assess the impact of the adjustment. Continuous variables were compared using t-tests. The significance level was set at $P < 0.05$. Analyses were carried out using Dr. SPSS II for Windows, standard version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Risk factors for recurrence in pathological stage I adenocarcinoma patients. Table 1 shows the risk factors for recurrence according to the clinicopathological characteristics of 1099 patients who had pathological stage I adenocarcinoma. In a univariate analysis, seven variables were found to be significantly associated with recurrence ($P < 0.05$): male sex; smoking

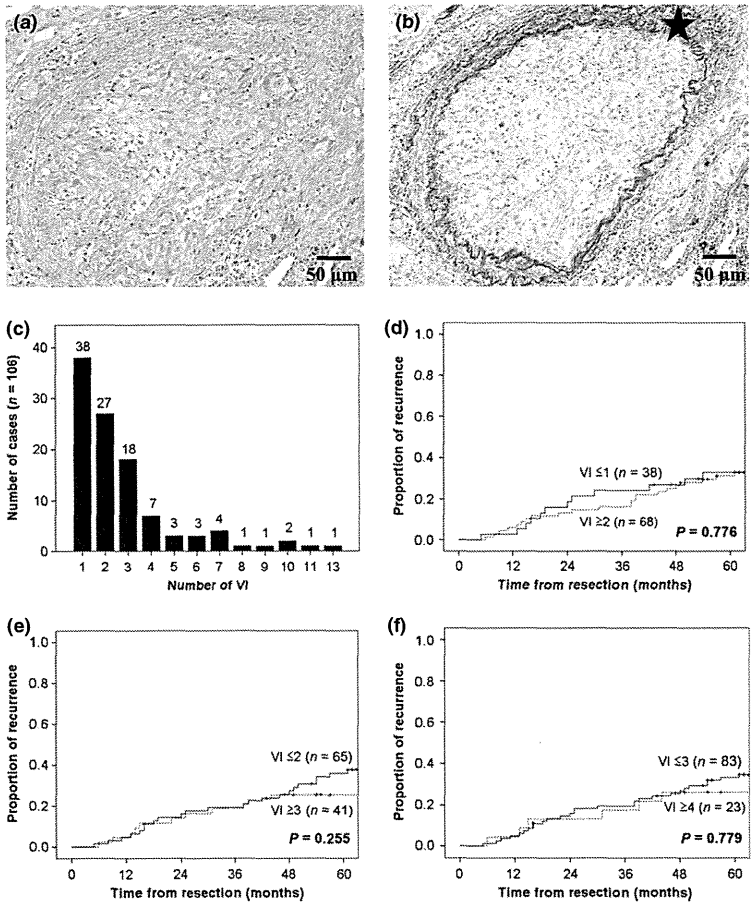

Fig. 2. (a,b) Representative images showing vascular invasion (VI) in lung adenocarcinoma patients. Sections were stained using H&E (a) and Victoria blue van Gieson (b). The star indicates elastic fibers with positive Victoria blue van Gieson staining. (c) Distribution of cases with VI according to the frequency of VI on the maximum cut surface. (d–f) Kaplan–Meier analyses of recurrence according to a VI cut-off level of 1 (d), 2 (e), and 3 (f).

Table 1. Univariate and multivariate analyses of risk factors for recurrence in patients with stage I adenocarcinoma

Variable	*n*	Univariate analysis HR (95% CI)	*P*-value†	Multivariate analysis HR (95% CI)	*P*-value‡
Age, years					
<65	533	1		1	
≥65	566	1.272 (0.954–1.697)	0.101	1.158 (0.858–1.561)	0.337
Sex					
Female	585	1		1	
Male	514	1.540 (1.156–2.052)	0.003*	1.069 (0.711–1.607)	0.748
Smoking habits					
Non-smoker	558	1		1	
Ever smoker	541	1.625 (1.217–2.171)	0.001*	1.158 (0.764–1.756)	0.488
CEA					
≤5	824	1		1	
>5	275	1.681 (1.243–2.273)	0.001*	1.026 (0.747–1.411)	0.872
Tumor size, cm					
≤3.0	860	1		1	
>3.0, ≤5.0	239	2.365 (1.758–3.182)	<0.001*	1.372 (0.985–1.912)	0.061
Histological differentiation					
Well/mod.	1010	1		1	
Poor	89	2.010 (1.319–3.063)	0.001*	0.765 (0.484–1.208)	0.250
Vascular invasion					
Absent	831	1		1	
Present	268	5.566 (4.165–7.439)	<0.001*	3.955 (2.818–5.552)	<0.001*
Pleural invasion					
Absent	906	1		1	
Present	193	4.152 (3.103–5.556)	<0.001*	2.136 (1.536–2.970)	<0.001*

*Significant. †Log–rank test. ‡Cox proportional hazard model. CEA, preoperative serum carcinoembryonic antigen level; CI, confidence interval; HR, hazard ratio; Poor, poorly differentiated carcinoma; Well/mod., well or moderately differentiated carcinoma.

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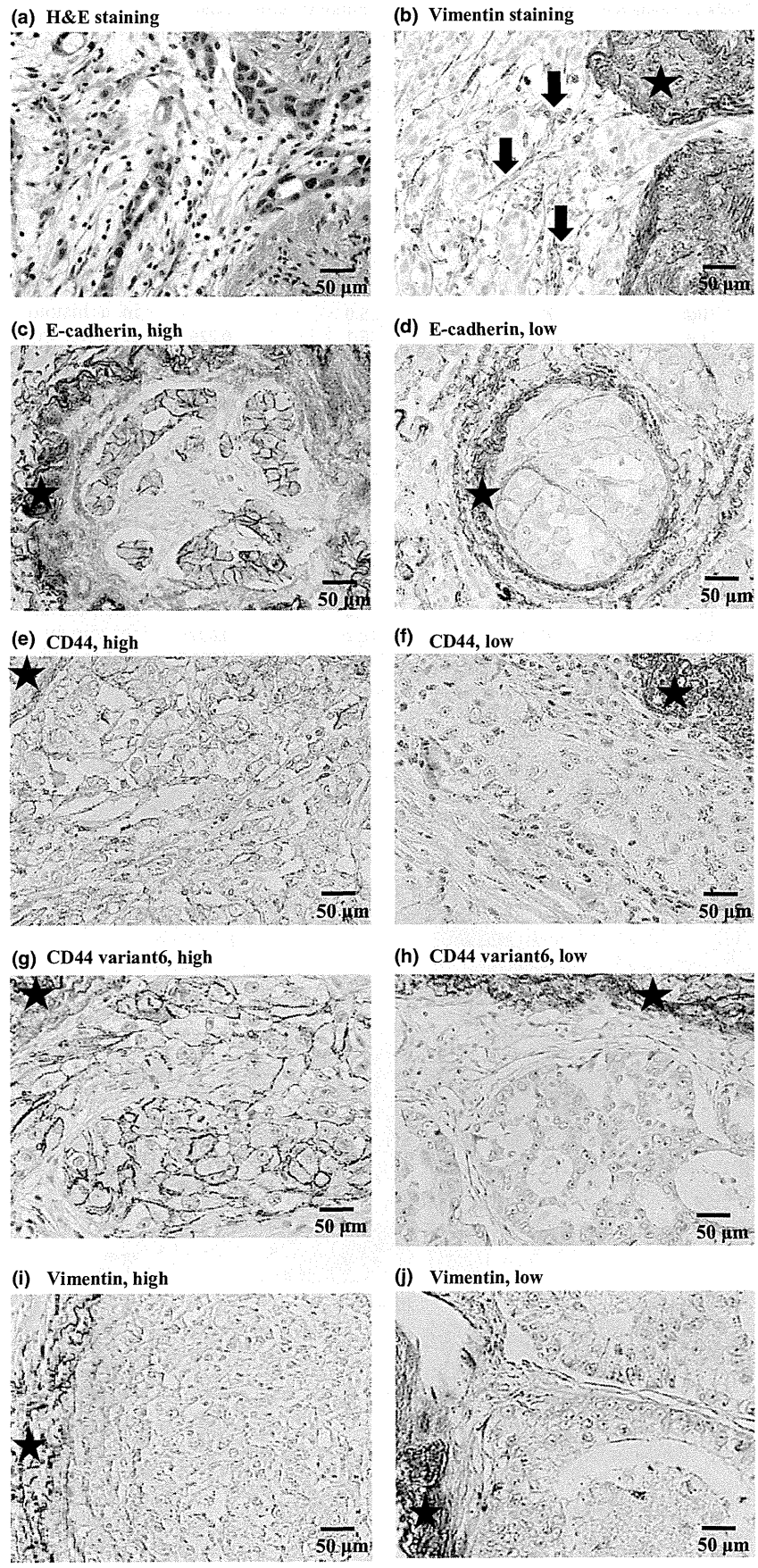

Fig. 3. Immunohistochemical staining of tumor tissue in patients with lung adenocarcinoma with intratumoral vascular invasion. Stars indicate elastic fibers with positive Victoria blue van Gieson staining. (a,b) Intravascular stromal cells stained with (a) H&E and (b) immunohistochemical staining for vimentin. The arrows point to round or spindle-shaped, brown-stained, non-cancerous cells. The staining results were negative for cancer cells. (c,d) E-cadherin expression in cancer cells. (e,f) CD44 expression in cancer cells. (g,h) CD44 variant 6 expression in cancer cells. (i,j) Vimentin expression in cancer cells.

Table 2. Five-year rates of recurrence in patients with stage I adenocarcinoma according to the immunohistochemical staining scores of intravascular tumors

	No. of patients	5-year PR, %	*P*-value†
Cancer phenotype			
E-cadherin			
High	20	55.0	
Low	21	9.5	0.004*
CD44			
High	19	42.1	
Low	22	22.7	0.410
CD44 variant 6			
High	20	25.0	
Low	21	38.1	0.229
Vimentin			
High	22	36.4	
Low	19	26.3	0.846
Stromal phenotype			
CD204			
High	20	50.0	
Low	21	14.3	0.016*
CD34			
High	22	50.0	
Low	19	10.5	0.007*
α-SMA			
High	19	47.4	
Low	22	18.2	0.033*

*Significant. †Log–rank test. α-SMA, α-smooth muscle actin; High, greater than the median; Low, less than or equal to the median; PR, proportion of recurrence.

history; elevated serum carcinoembryonic antigen value; large tumor size (3.0 ≥ 5.0 cm), poorly differentiated carcinoma; VI; and pleural invasion. A multivariate Cox proportional hazards model indicated that the presence of VI (HR = 3.955; $P < 0.001$) and pleural invasion (HR = 2.136; $P < 0.001$) were statistically significant predictors of recurrence.

Frequency of intratumoral vascular invasion is not a predictor of recurrence. To examine how VI features affect recurrence, we selected 106 patients without pleural invasion, another important predictive factor for recurrence, among 1099 patients (Fig. 1). In this cohort, age was the only variable capable of predicting recurrence.

First, we examined whether the frequency of intratumoral VI in a histological cross-section was a predictor of recurrence (Fig. 1). Figure 2(c) shows the distribution of cases according to the frequency of VI. The group with one VI contained the largest number of cases. Figure 2(d–f) shows Kaplan–Meier analyses of the rate of recurrence according to a cut-off level of 1, 2, and 3, respectively; however, no significant difference in the rate of recurrence between the VI-high group and the VI-low group was seen for each of the cut-off levels. These results indicate that the frequency of intratumoral VI is not a predictor of recurrence.

Correlations between immunophenotypes of cancer cells in blood vessels and rate of recurrence. As shown in Figure 3(a,b), the intravascular tumor tissue was composed of not only cancer cells, but also non-cancerous cells (arrows) that stained positive for vimentin, a marker of mesenchymal cells. We hypothesized that the microenvironment in the invaded blood vessels may be important for recurrence and examined the immunohistochemical properties of both cancer cells and non-cancerous cells

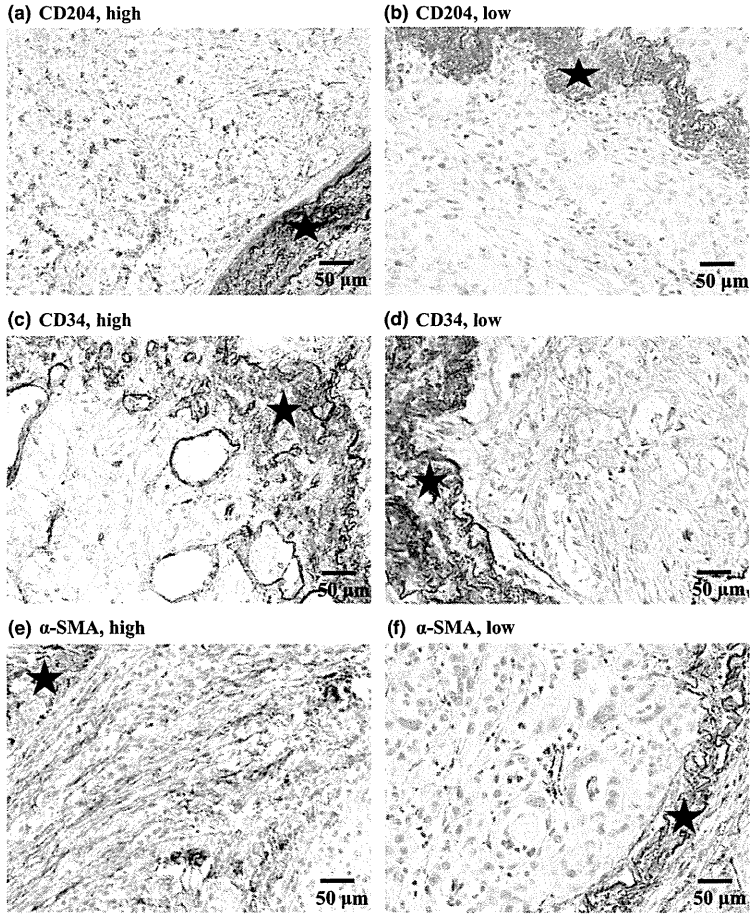

Fig. 4. Immunohistochemical staining of non-cancerous cells with intratumoral vascular invasion in patients with lung adenocarcinoma. (a,b) CD204 (+) macrophages; (c,d) CD34(+) microvessels; (e,f) α-smooth muscle actin (α-SMA) (+) myofibroblasts.

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existing in the blood vessels. As EMT has been considered important for the intravasation of cancer cells, we examined the expression levels of four EMT-related markers in cancer cells (Fig. 3c–j).[17] The median staining scores were 76 for E-cadherin, 92 for CD44, 0 for CD44 variant 6, and 0 for vimentin. The rates of recurrence in the high score (above median) and low score (below median) groups were 55.0 and 9.5 for E-cadherin, 42.1 and 22.7 for CD44, 25.0 and 38.1 for CD44 variant 6, and 36.4 and 26.3 for vimentin, respectively. The rate of recurrence was significantly higher in the group with the high E-cadherin score than in the group with the low E-cadherin score ($P = 0.004$), but there were no significant differences in the rates of recurrence observed between the high score and low score groups for CD44, CD44 variant 6, or vimentin ($P = 0.410$, $P = 0.229$, and $P = 0.846$, respectively; Table 2).

Correlations between number of activated macrophages, endothelial cells, and myofibroblasts in blood vessels and rate of recurrence. Next, we examined the numbers of activated macrophages (CD204+), microvessels (CD34+), and fibroblasts (α-SMA+) within the intravascular tumor tissue (Fig. 4). The median numbers of CD204(+) macrophages, CD34(+) microvessels, and α-SMA(+) myofibroblasts were 5.8, 1.7, and 3.6, respectively. The rates of recurrence in the high score and low score groups were 50.0 and 14.3 for CD204(+) macrophages, 50.0 and 10.5 for CD34(+) microvessels, and 47.4 and 18.2 for α-SMA(+) myofibroblasts, respectively. The rate of recurrence was significantly associated with a high number of CD204(+) macrophages ($P = 0.016$), CD34(+) microvessels ($P = 0.007$), and α-SMA(+) myofibroblasts ($P = 0.033$; Table 2).

Numbers of activated macrophages, endothelial cells, and myofibroblasts surrounding intravascular cancer cells with high or low scores for E-cadherin. We compared the numbers of infiltrated activated macrophages, endothelial cells, and myofibroblasts surrounding cancer cells with high or low scores for E-cadherin. The numbers of CD204(+) macrophages ($P = 0.033$) and α-SMA(+) myofibroblasts ($P = 0.011$) were significantly higher in the high E-cadherin score group than in the low E-cadherin score group. The number of CD34(+) microvessels ($P = 0.055$) tended to be higher in the high E-cadherin score group than in the low E-cadherin score group (Fig. 5).

Discussion

Vascular invasion reportedly represents the early phase of cancer metastasis and is an independent and statistically significant predictor of recurrence in many types of cancer.[4–6] However, not all patients with VI develop recurrences. First, we examined the frequency of intratumoral VI in a histological cross-section, but this parameter was not a predictor of recurrence. Therefore, we hypothesized that the microenvironment within the invaded blood vessels is important for recurrence. In the present study, we found that various stromal cells, including macrophages, endothelial cells, and fibroblasts, infiltrate and become a constituent of the "cancer tissue" in the invaded blood vessels, similar to the primary site. Immunohistochemical staining revealed that the rate of recurrence was significantly higher in patients with a high E-cadherin score for the cancer cells in the invaded blood vessels. In addition, the numbers of activated macrophages and fibroblasts (CD204 (+) macrophages and α-SMA(+) fibroblasts, respectively) and newly formed vessels (CD34(+) microvessels) within the intravascular tumor tissue were significantly higher among the patients with recurrences. As several studies have reported the significance of these cancer stromal cells within the primary tumor,[18–21] the current results imply that not only cancer cells, but also stromal cells may contribute to changes in the intravascular microenvironment and suggest that the tumor microenvironment created by cancer cells and stromal cells within the blood vessel is important for metastasis and recurrence. The present study is the first to indicate the possibility that the cancer microenvironment in invaded blood vessels has a considerable impact on recurrence.

Using animal models, Duda *et al.*[22] reported that tumor-associated stromal cells, such as fibroblasts, endothelial cells, or tumor-infiltrated myeloid cells, are shed from the primary tumor together with the accompanying cancer cells and survive in the blood circulation, proliferating within metastatic nodules. These findings suggest that not only the characteristics of floating cancer cells, but also those of circulating stromal cells, may affect the metastatic process. However, using surgically resected lung adenocarcinoma cases, Matsumura

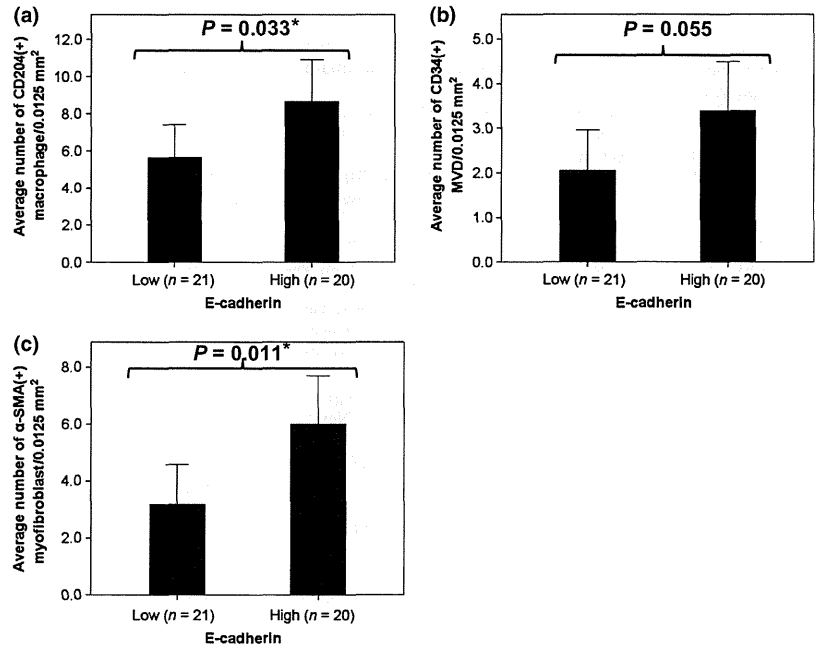

Fig. 5. Number of intravascular non-cancerous cells according to E-cadherin expression in cancer cells from patients with lung adenocarcinoma. (a) CD204(+) macrophages; (b) CD34(+) microvessels; (c) α-smooth muscle actin (α-SMA) (+) myofibroblasts.

et al.[10] revealed that CD204(+) macrophages infiltrate within and around the cancer nests in permeated lymphatic vessels and that a large number of infiltrating CD204(+) macrophages is correlated with the presence of pulmonary metastasis. These results suggest the importance of the microenvironment in the invaded blood and or lymphatic vessels.

Epithelial–mesenchymal transition has been recognized as a phenomenon during which tumor cells in the primary lesion invade the surrounding stroma. Many researchers have postulated that the loss or repression of E-cadherin is strongly linked with cancer invasiveness, metastasis, and patient prognosis.[23,24] However, contradictory results have also been reported. Imai *et al.*[25] reported that E-cadherin expression in ovarian carcinoma cells at metastatic sites of peritoneal dissemination is higher than that in the primary ovarian tumor. Moreover, the expression of E-cadherin in intralymphatic cancer cells was significantly elevated in a group of patients that showed larger numbers of pulmonary metastases,[10] consistent with the results of the present study. Although the function of E-cadherin is essential during the metastatic process, its role may differ between cancer cells located inside and those located outside blood vessels.

The present study revealed that higher numbers of stromal cells, such as CD204(+) macrophages, CD34(+) microvessels, and α-SMA(+) myofibroblasts, are present together with cancer cells with high levels of E-cadherin in the tumor tissue within the invaded blood vessel. This phenomenon can be explained in two possible ways: (i) cancer cells expressing high levels of E-cadherin may carry more stromal cells when they invade the blood vessels; and (ii) cancer cells expressing high levels of E-cadherin may attract more stromal cell precursors in the blood circulation after vascular invasion has occurred.[26] These possible molecular mechanisms will be the subjects of future examinations.

The present study examined tumor specimens from patients with lung adenocarcinoma. Whether these results are applicable to other types of cancer, such as squamous cell carcinoma or neuroendocrine carcinoma, will be the topic of future investigations.

In conclusion, this study showed that the presence of histological VI with abundant stromal cell infiltrates may predict a relatively high likelihood of recurrence and suggested that the microenvironment of the invaded blood vessels inside primary tumors might be capable of predicting the likelihood of metastasis in patients with pathological stage I adenocarcinoma. A more mechanistically oriented experimental approach is required to clarify the key regulators of the intravascular microenvironment, possibly leading to useful therapeutic options in the future.

Acknowledgments

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Disclosure Statement

The authors have no conflict of interest.

Abbreviations

α-SMA	α-smooth muscle actin
CEA	carcinoembryonic antigen
CI	confidence interval
EMT	epithelial–mesenchymal transition
HR	hazard ratio
NSCLC	non-small-cell lung cancer
VI	vascular invasion
VVG	Victoria blue van Gieson

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Identification of Early T1b Lung Adenocarcinoma Based on Thin-Section Computed Tomography Findings

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Introduction: The aim of this study was to radiologically identify early lung adenocarcinoma in clinical T1bN0M0 lung cancer, based on pathological findings and long-term prognosis.

Methods: In this study, we reviewed lung nodules findings on thin-section computed tomography in 173 patients with clinical T1bN0M0 lung adenocarcinoma who underwent surgery between 2003 and 2007. The ratio of the size of solid attenuation to the maximum tumor dimension (consolidation/tumor [C/T] ratio) was calculated. We defined two groups of patients by C/T ratio cutoff levels of 0.00, 0.25, 0.50, 0.75, and 1.00 and compared the rates of pathological nonaggressive lung adenocarcinoma, overall survival, and recurrence rates between the groups. The percentages of predominant histological subtypes were compared between two groups divided by the optimal cutoff level. Various clinical factors were analyzed by univariate and multivariate analyses to predict pathological lymph node involvement.

Results: The median follow-up period was 62 months. All patients with C/T ratios of 0.5 or less were diagnosed as having pathological nonaggressive adenocarcinomas, and there was no recurrence; their 5-year overall survival rate was 97.4%, which was significantly better than that for patients with C/T ratios of greater than 0.5 (76.2%). None of the ground-glass opacity–predominant tumors were predominantly solid adenocarcinoma with mucin. The C/T ratio of 0.5 or more was an independent predictor of lymph node involvement.

Conclusion: In patients with clinical T1bN0M0 disease, the C/T ratio of 0.5 or less identified early lung adenocarcinoma. In patients with the identified early disease, a feasibility study of limited surgery may be warranted.

Key Words: Early lung cancer, Ground-glass opacity, Non–small-cell lung cancer, Limited surgery.

(*J Thorac Oncol.* 2013;8: 1289–1294)

Remarkable improvement in spatial resolution of computed tomography (CT) has provided new tools for lung cancer diagnosis. Thin-section CT (TSCT) findings of lung cancer have been extensively analyzed. Various measures to predict less-invasive lung cancers have been reported, for example, classically used tumor size, tumor shadow disappearance rate,[1,2] visual estimation of the consolidation component,[3–5] size of the solid component, maximum standardized uptake value by ^{18}F-fluorodeoxyglucose positron emission tomography/CT,[6] and the ratio of the size of solid attenuation to the maximum tumor dimension (consolidation/tumor [C/T] ratio).[7] Suzuki et al.[8] reported a multi-institutional prospective radiological study for early lung cancer (Japan Clinical Oncology Group: JCOG trial 0201) in 2011 and concluded that the C/T ratio was the most reliable measure for identification of nonaggressive lung cancer on TSCT, compared with the visual estimation and tumor shadow disappearance rate methods. On the basis of these results, two pivotal prospective studies on limited resection for small peripheral lung cancer assessed by C/T ratios were initiated in Japan (JCOG trial 0802/West Japan Oncology Group trial 4607L and JCOG trial 0804).[9] In addition, there is an ongoing, prospective, randomized controlled trial (lobectomy versus sublobar resection) for small (≤2 cm) peripheral stage IA non–small-cell lung cancer in the United States of America and Canada (Cancer and Leukemia Group B trial 14053). However, there are no prospective, limited resection trials for clinical T1b (cT1b) early lung cancer. The aim of this study was to radiologically identify early lung adenocarcinoma in patients with cT1bN0M0 lung cancer, based on pathological findings and long-term outcomes.

PATIENTS AND METHODS

Patient Selection

Between January 2003 and December 2007, 1000 non-small cell lung cancer patients underwent complete resection by lobectomy or pneumonectomy with systematic lymph node dissection. Basically, regional lymph nodes were dissected in our hospital according to the previous article by Naruke et al.[10] Of these, 210 patients (21.0%) had lung adenocarcinoma of cT1bN0M0 according to the 7th edition of the tumor–node–metastasis (TNM) classification.[11] We excluded patients with multiple primary lung cancers, extensive cavity formation, or massive occlusive pneumonia on preoperative TSCT as well as those who underwent induction therapy or whose clinical course records were incomplete. Thus, we included 173 patients (17.3%) whose preoperative TSCT data

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were available for review in this study. Data collection and analyses were approved, and the need to obtain informed consent from each patient was waived by the institutional review board in February 2013.

Radiological Evaluation by TSCT

Contrast-enhanced CT scans at 5 to 10 mm collimation of the chest and upper abdomen were used to assess the clinical staging of all patients with cT1N0M0 lung cancer. In addition, TSCT images at 1 to 2 mm collimation were used to evaluate primary lesions. The X-vigor CT system (Toshiba Medical Systems, Tokyo, Japan) was used to perform the CT scans. CT images were evaluated on a monitor display with a window level of −600 HU and a window width of 1800 HU. Two observers (KA and TH), who were unaware of the pathological findings and prognosis, reviewed each lung nodule on preoperative TSCT scans. Tumor diameter was measured at the longest dimension of a tumor. The C/T ratio was calculated in one dimension on TSCT (Supplemental Figure). The consolidation component was defined as an area of increased opacification that completely obscured the underlying vascular structures. Ground-glass opacity (GGO) was defined as increased hazy density in an area without obscuring the underlying vascular structure.[8] Discrepancies in interpretation between observers were resolved by consensus. We set C/T ratio cutoff levels at 0.0, 0.25, 0.5, 0.75, and 1.0. A C/T ratio of 0.0 represented pure GGO nodules, and the C/T ratio of 1.0 represented solid nodules without GGO components. Disease stage during the study period was determined according to the 6th edition of the TNM classification,[12] but for this report we reclassified this study population according to the 7th edition of the TNM classification.[11]

Pathological Evaluation

Surgical specimens were fixed with 10% formalin and embedded in paraffin. Serial 4-μm sections were stained with hematoxylin and eosin and by the Alcian blue–periodic acid–Schiff method for cytoplasmic mucin production. We used elastica van Gieson or Victoria blue–van Gieson staining to visualize elastic fibers and evaluate vascular and pleural invasion. Lymphatic permeation was diagnosed with the help of D2-40 staining. Histological type was determined according to the World Health Organization classification of cell types.[13]

Patient Follow-Up

Patients were evaluated at 3-month intervals for the first 2 years and typically at 6-month intervals thereafter on an outpatient basis. The follow-up evaluation included physical examination, chest radiography, and blood examination including pertinent tumor markers. Whenever any symptoms or signs of recurrence were detected, further evaluations were conducted, including CT scan of the chest and abdomen, brain magnetic resonance imaging, and bone scintigraphy. After 2004, integrated positron emission tomography/CT was also performed when appropriate. Annual follow-up CT examination was also used for lung cancer patients who had undergone complete resection in the absence of some symptoms or abnormal examination findings. Recurrence was diagnosed on the basis of compatible physical examination and diagnostic imaging findings, and the diagnosis was histologically confirmed when clinically feasible. The length of overall survival was defined as the interval in months between the date of surgical intervention and the last follow-up date or death resulting from any cause. Observations were censored at the last follow-up when the patient was alive or lost to follow-up. The date of recurrence was defined as the date of histological proof or, in cases diagnosed on the basis of clinicoradiological findings, the date of identification by a physician.

Pathological and Prognostic Analyses

We defined pathological nonaggressive lung adenocarcinoma as lung adenocarcinoma without nodal involvement, vascular invasion, lymphatic permeation, and pleural invasion, and its rates, overall survival, and recurrence rates were compared between each group defined by the C/T ratio cutoff levels. On the basis of the pathological findings and prognoses, we determined the optimal cutoff C/T ratio that best discriminated early lung adenocarcinoma. The predominant histological subtype (bronchioloalveolar carcinoma [BAC], papillary adenocarcinoma, acinar adenocarcinoma, and solid adenocarcinoma with mucin) of each tumor was determined, and the percentages of these subtypes were calculated for the groups divided by the optimal C/T ratio cutoff level. Various clinical factors (C/T ratio, sex, age, smoking history, preoperative serum carcinoembryonic antigen level, laterality, tumor origin lobe, and maximum tumor diameter) were used for univariate and multivariate analyses to predict pathological lymph node involvement.

Statistical Analysis

All cumulative overall survival rates were estimated by the Kaplan–Meier method. Comparisons between groups were calculated by the log-rank test. We used Fisher's exact test for univariate analysis as well as multivariate logistic regression analysis to identify pathological lymph node involvement predictors. All statistical analyses were performed using JMP 10 statistical software (Version 10.0.2. 64-bit edition; SAS Institute Inc., Cary, NC) and GraphPad Prism (Prism for Windows, Version 5.02; GraphPad Software, Inc., La Jolla, CA).

RESULTS

Patient clinical and pathological characteristics are summarized in Table 1. The median follow-up period was 62 months (range, 3–108 months). The average ± SD of the tumor diameter was 25 ± 2.8 mm. There were only five patients (2.9%) with pure GGO tumor (C/T ratio = 0.0) and 69 patients (39.9%) with completely solid tumor (C/T ratio = 1.0). There were no significant correlations between the maximum tumor diameter and C/T ratio ($r = 0.16$, Spearman's rank correlation coefficient). Lymph node metastasis was pathologically confirmed in 19 patients (10.9%).

There were 109 patients (63.0%) with pathological nonaggressive lung adenocarcinoma (defined as lung adenocarcinoma without nodal involvement, vascular invasion, lymphatic permeation, and pleural invasion). All patients with C/T ratios

TABLE 1. Patients Clinicopathological Characteristics

Patient Characteristics	No. of Patients (%) (N = 173)
Age (yr)	
<70	111 (64.2)
≥70	62 (35.8)
Sex	
Male	75 (43.4)
Female	98 (56.6)
Smoking history	
Never smoker	85 (49.4)
Current/former smoker	88 (50.6)
Preoperative serum CEA level[a]	
≤5.0 ng/ml	137 (79.2)
>5.0 ng/ml	35 (20.2)
Tumor location	
Right upper lobe	62 (35.8)
Right middle lobe	13 (7.5)
Right lower lobe	32 (18.5)
Left upper lobe	41 (23.7)
Left lower lobe	25 (14.5)
Maximum tumor size (mm)	
Average ± SD	25 ± 2.8
C/T ratio	
0 (pure GGO)	5 (2.9)
>0, ≤0.25	8 (4.6)
>0.25, ≤0.5	26 (15.0)
>0.5, ≤0.75	33 (19.1)
>0.75, <1.0	32 (18.5)
1 (solid tumor)	69 (39.9)
Pathological lymph node metastasis	
pN0	154 (89.0)
pN1	9 (5.2)
pN2	10 (5.7)
Lymphatic permeation	
Absent	147 (85.0)
Present	26 (15.0)
Vascular invasion	
Absent	123 (71.1)
Present	50 (28.9)
Pleural invasion	
Absent	138 (80.2)
Present	34 (19.8)

[a]One patient with missing value.
CEA, carcinoembryonic antigen; C/T ratio, consolidation/tumor ratio; GGO, ground-glass opacity.

of 0.5 or less had pathological nonaggressive cancers (Table 2). Of the 69 pure solid tumors (C/T ratio = 1.0), 38 (55%) were pathologically diagnosed as invasive adenocarcinoma (having any of the following pathological findings: nodal involvement, vascular invasion, lymphatic permeation, or pleural invasion). When C/T ratio cutoff levels were set at 0.50, 0.75, and 1.00, overall survival was significantly better in the lower C/T ratio group than that in the higher C/T ratio group (Fig. 1). The

TABLE 2. Relationship between C/T Ratio and Pathological Invasiveness

C/T Ratio	Total No. of Patients	Pathological Nonaggressive Cancer[a]	Pathological Invasive Cancer[a]
0 (Pure GGO)	5	5 (100)	0 (0)
0 < C/T ratio ≤ 0.25	8	8 (100)	0 (0)
0.25 < C/T ratio ≤ 0.5	26	26 (100)	0 (0)
0.5 < C/T ratio ≤ 0.75	33	24 (73)	9 (27)
0.75 < C/T ratio < 1.0	32	15 (47)	17 (53)
1 (Pure solid)	69	31 (45)	38 (55)

Numbers in parentheses are percentages.
[a]Defined as absence of nodal involvement, pleural invasion, lymphatic permeation, and vascular invasion. If one or more of these findings were positive, tumors were classified as invasive cancer.
C/T ratio, consolidation/tumor ratio; GGO, ground-glass opacity.

5-year survival rate of patients with C/T ratios of 0.5 or less was 97.4%, which was significantly better compared with that of the patients with C/T ratios 0.5 or more (76.2%; Fig. 1*C*). During the follow-up period, 42 recurrences (22 locoregional only and 20 including distant sites) were observed (Table 3). Patients with C/T ratios of 0.5 or less did not develop recurrence, but one patient died of another cancer. In contrast, 31% patients (42 of 134) with C/T ratios 0.5 or more developed recurrences. On the basis of these results, we determined that a C/T ratio cutoff level of 0.5 was optimal for discrimination of early lung adenocarcinoma. Otherwise, seven patients (6%) among 109 patients who revealed pathological nonaggressive lung adenocarcinoma developed recurrence and 31 patients (48%) among 64 patients who revealed pathological invasive lung adenocarcinoma developed recurrence.

In more than half of the GGO-predominant tumors (C/T ratio ≤0.5), the predominant histological subtype was BAC (62%) followed by papillary adenocarcinoma (33%), whereas there were no tumors of predominantly solid adenocarcinoma with mucin. In contrast, of all consolidation-predominant tumors (C/T ratio >0.5), solid adenocarcinomas accounted for 11% (16 of 152) and BAC for only 22% (33 of 152; Table 4). There was a significant difference in the distribution of predominant subtypes between GGO-predominant and consolidation-predominant tumors ($p < 0.01$; Fisher's exact test).

On the univariate analysis, the C/T ratio cutoff levels of 0.0, 0.25, and 1.0 were not significantly associated with pathological lymph node metastasis. On multivariate analysis, the C/T ratio cutoff level of 0.75 was an independent predictor of node metastasis (odds ratio for C/T ratio >0.75, 3.4); however, a C/T ratio cutoff level of 0.5 was by far a stronger independent predictor (odds ratio for C/T ratio >0.5, >100; Table 5) than C/T ratio of 0.75.

DISCUSSION

Since 1995, lobectomy and lymph node dissection have been the standard therapy for stage IA lung cancer, because of the only randomized controlled trial reported by the Lung Cancer Study Group.[14] However, the development of CT technology and the widespread use of TSCT have enabled earlier

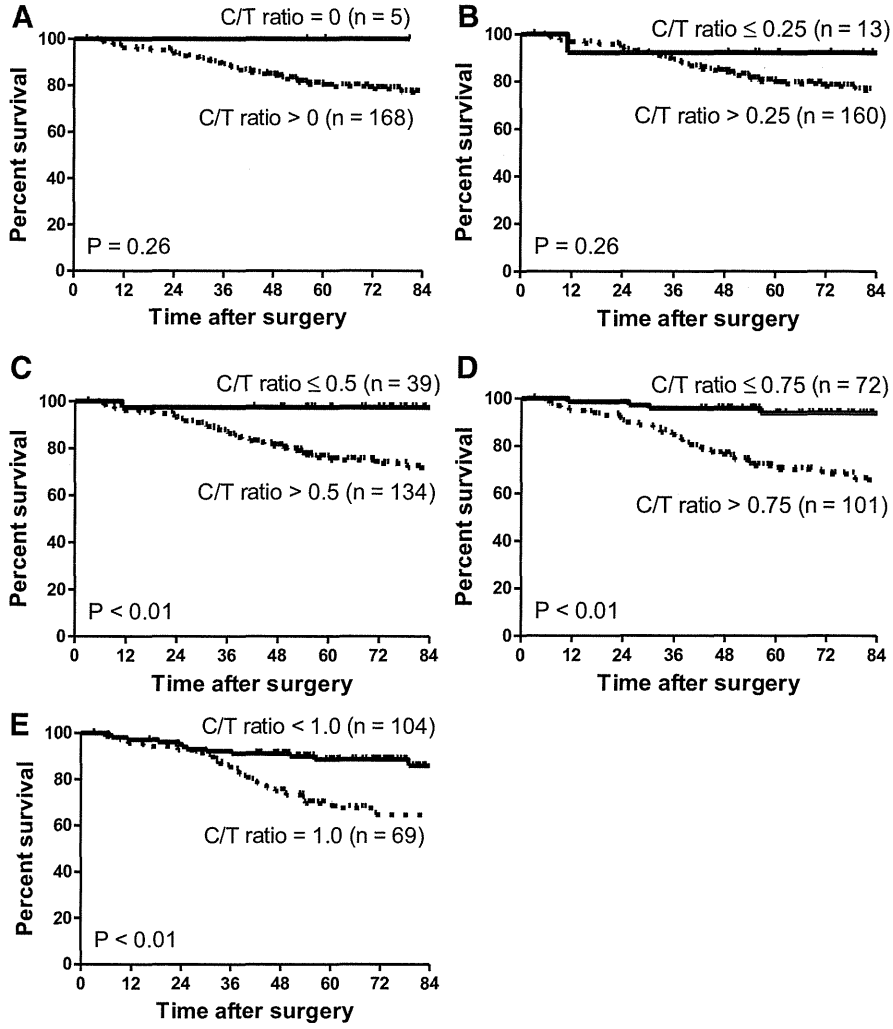

FIGURE 1. Overall survival comparison between two groups divided by C/T ratio. *A*, Cut-off level of C/T ratio at 0, (*B*) at 0.25, (*C*) at 0.5, (*D*) at 0.75, and (*E*) at 1.0. *p* was determined by the log-rank test. C/T, consolidation/tumor.

detection of primary lung cancers, particularly tumors with GGO components. Many retrospective studies[3–5,7,15–18] have focused on identifying less-invasive lung cancers on the basis of preoperative TSCT findings along with the recognition of BAC and Noguchi's classification.[16,19] On the basis of the findings from these retrospective studies, two trials of limited resection for small peripheral lung cancer according to C/T ratios determined on preoperative TSCT were initiated for patients with T1a lung cancer in Japan.[7,8,15,17] The results from these studies may change future surgical strategies for lung adenocarcinomas that are less than 2 cm in diameter.

In 2009, the TNM classification for primary lung cancer was revised, and T1 tumors were subcategorized as T1a (≤2.0 cm) and T1b (>2.0 cm and ≤3.0 cm) in the current 7th

TABLE 3. Relationship between Relapse Site and C/T Ratio

C/T Ratio	Total No. of Patients	No Recurrence	Locoregional Site Only	Including Distant Site
0 (Pure GGO)	5	5 (100)	0 (0)	0 (0)
0 < C/T ratio ≤ 0.25	8	8 (100)	0 (0)	0 (0)
0.25 < C/T ratio ≤ 0.5	26	26 (100)	0 (0)	0 (0)
0.5 < C/T ratio ≤ 0.75	33	27 (82)	4 (12)	2 (6)
0.75 < C/T ratio < 1.0	32	21 (66)	7 (22)	4 (13)
1 (Pure solid)	69	44 (64)	11 (16)	14 (20)

Numbers in parentheses are percentages.
C/T ratio, consolidation/tumor ratio; GGO, ground-glass opacity.

TABLE 4. Predominant Histological Subtype

C/T Ratio	C/T ≤0.5 (%) *n* = 39	0.5 < C/T < 1.0 (%) *n* = 83	C/T = 1.0 (%) *n* = 69
Bronchioloalveolar carcinoma	21 (62)	16 (19)	17 (25)
Papillary adenocarcinoma	13 (33)	38 (46)	32 (46)
Acinar adenocarcinoma	2 (5)	22 (27)	11 (16)
Solid adenocarcinoma with mucin	0 (0)	7 (8)	9 (13)

C/T ratio, consolidation/tumor ratio.

TABLE 5. Predictive Factor for Pathologic Lymph Node Metastasis

Variables	Univariate Analysis[a] *p* Value	Multivariate Analysis[b] Odds Ratio	95% CI	*p* Value
Sex (man)	0.46	3.53	0.72–18.77	0.12
Age (≥70)	1.00	1.07	0.38–3.26	0.90
Smoking history (smoker)	0.47	4.39	0.92–24.09	0.06
Preoperative serum CEA level (>5.0 ng/ml)	0.03	2.98	1.00–8.74	0.05
Tumor laterality (right/left)	0.80	NA	NA	NA
Tumor location (RUL/RML/RLL/LUL/LLL)	0.78	NA	NA	NA
Larger maximum tumor size (mm)	0.79	NA	NA	NA
C/T ratio (>0.5)	<0.01	>100	2.86-	<0.01

[a]Fisher's exact test or logistic analysis.
[b]Logistic regression analysis.
CI, confidence interval; CEA, carcinoembryonic antigen; NA, not applicable; RUL, right upper lobe; RML, right middle lobe; RLL, right lower lobe; LUL, left upper lobe; LLL, left lower lobe; C/T ratio, consolidation/tumor ratio.

TNM classification. Because most researchers have targeted peripheral early lung adenocarcinomas of 2 cm or less in diameter, we focused on T1b tumors and examined the possibility of limited resection for this population.

Aoki et al.[15] reported that patients who had tumors composed of less than 50% GGO showed significantly better outcomes than patients who had tumors composed of less than 50% GGO. They also pointed out that 17 patients with adenocarcinomas (<2 cm) that were composed of less than 50% GGO had no lymph node metastases or vessel invasion and developed no recurrence. However, the median follow-up period in that study was relatively short at 31 months, and the study did not separately examine cT1b patients.[15]

The present study showed that GGO-predominant tumors (C/T ratio ≤0.5) in patients with cT1b lung adenocarcinoma were nonaggressive adenocarcinomas, and no locoregional or distant metastases developed after lobectomy and lymph node dissection. Patients were observed for a minimum of 5 years. The result that no distant or lymph node metastases were observed in the GGO-predominant tumors strongly suggests that these tumors are localized. These results are compatible with those from a recent report on a prospective CT finding study in Japan to define nonaggressive adenocarcinoma of the lung.[20] These GGO-predominant tumors can most likely be cured by limited lung resection alone without lymph node dissection when an adequate margin is secured.

GGO components of lung tumors on TSCT histologically correspond to lepidic growth patterns of cancer cells, and central consolidation typically corresponds to alveolar collapse and/or fibrotic foci.[16,21] Kuriyama et al. reported that the measurement of the GGO area in a lung tumor was useful in differentiating localized BAC from invasive adenocarcinoma.[21] It may be difficult to clearly determine histological subtypes of adenocarcinoma on the basis of TSCT findings, but we found that no cT1b GGO-predominant (C/T ratio ≤0.5) lung adenocarcinomas were solid adenocarcinoma predominant. Solid adenocarcinoma with mucin is known to be highly aggressive, with frequent vessel invasion and lymph node metastases, and their surgical outcome is significantly poorer than that for other subtypes.[22] In consolidation-predominant (C/T ratio >0.5) tumors, solid adenocarcinomas with mucin were histologically predominant in approximately 10% cases. Patients with these tumors must be carefully evaluated before deciding whether limited resection or omission of lymph node dissection is indicated.

A limitation of this study is its retrospective nature. Because the C/T ratio is a subjective parameter, more objective and quantitative parameters of these nodules are sought. Further research is necessary to achieve good inter- and intraobserver reproducibility of C/T ratio. de Hoop et al.[23] reported mass value, which was calculated by multiplying nodule volume by mean nodule density on TSCT as an objective measurement. In the future, innovative developments in imaging technology may enable accurate diagnosis of less-invasive lung cancer comparable with pathological evaluation.

In conclusion, the C/T ratio of 0.5 or less identified early lung adenocarcinoma in patients with clinical T1bN0M0 disease. None of these tumors were solid adenocarcinoma predominant. The patients with these tumors had no lymph node involvement and developed no recurrence. These findings warrant a feasibility study of sublobar resection or reduction of the extent of mediastinal lymph node dissection for this population.

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Visceral Pleural Invasion Classification in Non–Small-Cell Lung Cancer in the 7th Edition of the Tumor, Node, Metastasis Classification for Lung Cancer: Validation Analysis Based on a Large-Scale Nationwide Database

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Objective: In the 7th tumor, node, metastasis (TNM) classification, visceral pleural invasion (VPI) is defined as invasion beyond the elastic layer, including invasion to the visceral pleural surface, and T1 tumors with VPI are upgraded to T2a. To validate this, we analyzed the survival of non–small-cell lung cancer patients from a nationwide database and evaluated the prognostic impact of VPI.

Methods: The clinicopathological characteristics and prognosis of 4995 patients who were included in the registry study of the Japanese Joint Committee of Lung Cancer Registry were retrospectively analyzed with a special interest in the prognostic impact of VPI. These patients underwent surgery in 2004 and were pathologically staged as T1a-3N0. VPI was defined as including PL1 and PL2 according to the 7th TNM Classification, but the Japanese Joint Committee of Lung Cancer Registry did not collect data regarding staining or how extensively VPI was evaluated in each participating institution.

Results: The survival differences were statistically significant between PL0 and PL1, PL1 and PL2, as well as PL2 and T3. There were no significant survival differences between T1a with VPI and T1b without VPI, or between T1a with VPI and T2a without VPI. There were no significant survival differences between T1b with VPI and T2a without VPI, or between T1b with VPI and T2b without VPI. There were no significant survival differences between T2a with VPI and T2b without VPI, or between T2b with VPI and T2b without VPI. T3 showed significantly worse prognosis than T2a with VPI and T2b with VPI.

Conclusions: In addition to the current TNM classification recommendations, in which T1 tumors with VPI are upgraded to T2a, T2a tumors with VPI should be classified as T2b.

Key Words: TNM classification, NSCLC, visceral pleural invasion

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Visceral pleural invasion (VPI) of lung cancer has been known to be a poor prognostic factor.[1–10] In the 7th edition of the tumor, node, metastasis (TNM) classification for lung cancer, pleural invasion status is classified as follows: PL0, tumor within the subpleural lung parenchyma or superficial invasion into the pleural connective tissue beneath the elastic layer; PL1, tumor invasion beyond the elastic layer; PL2, tumor invasion to the pleural surface; and PL3, tumor invasion into any part of the parietal pleura.[11,12] Although the current TNM classification does not describe a survival difference between PL1 and PL2[11,12], VPI is defined to include PL1 and PL2. Tumors of 3 cm or less (T1a and T1b) with VPI (PL1 and PL2) are upgraded to T2a, whereas tumors greater than 3 and 7 cm or less (T2a and T2b) with VPI remain unchanged as T2.[13] These recommendations—to upgrade the T-classification according to VPI status—were based on the results of five retrospective studies[1–3,8,14] and not on the large-scale data accumulated by the International Association for the Study of Lung Cancer (IASLC) Lung Cancer Project.[11]

In 2009, 253 Japanese institutions submitted information to the Japanese Joint Committee of Lung Cancer Registry (JJCLCR) regarding the outcome and clinicopathologic profiles of patients who had undergone surgical resection for primary lung cancer in the year 2004.[15] We retrospectively analyzed the survival of almost 5000 patients with pulmonary non–small-cell lung cancer (NSCLC) without node involvement from this registration to evaluate the impact of VPI on survival, and we

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propose incorporating VPI into T-status classification in the forthcoming TNM classification of the Union for International Cancer Control (UICC) staging system.

PATIENTS AND METHODS

Patient Cohort

As described previously, the JJCLCR performed a nationwide retrospective registry study in 2010 on the outcome and clinicopathologic profiles of resected primary lung neoplasms in Japan.[15] Only primary lung cancers that had been resected in 2004 at certified teaching hospitals in Japan, with a follow-up period of at least 5 years, were considered eligible for the registration. The committee received the registered data of 11,663 patients from 253 teaching hospitals. The registry questionnaire included the following items: (1) demographic background, (a) date of registry, (b) sex, (c) birth month and year, and (d) date of diagnosis; (2) preoperative status, (a) Eastern Cooperative Oncology Group performance status, (b) preoperative comorbidity, (c) smoking status, and (d) status of serum tumor markers (CEA, SCC or CYFRA, SLX and NSE, or Pro-GRP); (3) clinical T factors, (a) tumor size, (b) extent of invasion to the main bronchus, (c) pleural invasion, (d) intrapulmonary metastasis, (e) status of pleural effusion, (f) extent of atelectasis, and (g) status of invaded organ; (4) clinical N factor (status of removal of and metastasis to each lymph node); (5) clinical M factor (metastasized organ); (6) type of surgery, (a) induction therapy, (b) extent of lung resection, (c) place of tumor origin, (d) extent of lymph node removal, (e) gross curative status, (f) status of residual tumor, (g) lavage cytology findings, and (h) combined resection; (7) postoperative morbidity; (8) tumor histology; (9) adjuvant therapy; (10) pathological T factors, (a) tumor size, (b) extent of bronchial involvement, (c) pleural invasion, (d) intrapulmonary metastasis, (e) status of pleural effusion, (f) pleural dissemination, (g) status of atelectasis, and (h) status of invaded organ; (11) pathological N factor (status of removal of and metastasis to each lymph node); and (12) pathological M factor (metastasized organ). The extent of resection (exploratory, R0, R1, or R2) was also registered. Although the Japan Lung Cancer Society also recommends using not only hematoxylin and eosin (HE) staining but also elastic staining such as Victoria-blue van Gieson staining in VPI evaluation, the JJCLCR did not collect data regarding staining or how extensively VPI was evaluated in each participating institution. Diseases were staged based on the 7th edition of the UICC TNM classification.[11,12] Histopathologic classifications were described according to World Health Organization criteria.[16] Recurrent or multiple lung cancers were not included in the registration.

Of the 11,663 patients, 4995 patients (42.8%) underwent pulmonary resection (lobectomy or greater) and systematic mediastinal lymph node dissection for pathologically T1aN0, T1bN0, T2aN0, T2bN0, or T3N0 NSCLC. All these patients had curative resection, which was defined as complete removal of the ipsilateral hilar and mediastinal lymph nodes together with the complete resection of the primary tumor. Patients who had induction chemotherapy, radiotherapy, or both, and patients with evidence of residual tumor at the surgical margin, malignant effusion, interlobar invasion, or distant metastasis, verified intraoperatively or by means of postoperative pathologic examination were excluded from this study.

Statistical Analysis

Pleural invasion status was classified according to the 7th edition of the UICC TNM classification[11–13]: PL0, tumor within the subpleural lung parenchyma or superficial invasion into the pleural connective tissue beneath the elastic layer; PL1, tumor invasion beyond the elastic layer; PL2, tumor invasion to the pleural surface; and PL3, tumor invasion into any part of the parietal pleura. In the following descriptions, T-classification is determined excluding VPI status, but PL3 tumors are classified as T3.

First, we analyzed the overall survival of PL0, PL1 and PL2 or T3 patient groups. Second, defining VPI to include PL1 and PL2, we analyzed the overall survival of the pT1a patient groups with or without VPI, pT1b with or without VPI, pT2a with or without VPI, and pT2b with or without VPI or T3. The follow-up period was defined as the time from the date of surgery to the most recent follow-up examination. The survival period was defined as the number of months from the day of surgery to the day of death from any cause. Survival curves were estimated using the Kaplan-Meier method. Differences in survival were tested using the log-rank test. A p value of less than 0.05 was considered to indicate a statistically significant difference. All statistical analyses were performed using software packages (SAS version 9.1.3 [SAS Institute, Inc., Cary, NC], SPSS version 19 [IBM Corp., New York, NY]).

This study was approved by the institutional review board of Osaka University Medical Hospital, where the office of JJCLCR is located, on August 13, 2009 (approval no. 09124).

RESULTS

Patient Characteristics and Visceral Pleural Invasion

Table 1 shows the patient characteristics. There were 2981 men and 2014 women, aged 15 to 90 years (median, 67 years). The extent of pulmonary resection was pneumonectomy (n = 65), bilobectomy (n = 122), and lobectomy (n = 4808). The histological types were adenocarcinoma (n = 3638), squamous cell carcinoma (n = 1028), adenosquamous carcinoma (n = 84), large-cell carcinoma (n = 149), and other histological types (n = 96).

Survival Differences

The overall 5-year survival rates for PL0 (n = 3606), PL1 (n = 727), PL2 (n = 219), and T3 (n = 443) patients were 87%, 77%, 69%, and 54%, respectively. There were significant survival differences between PL0 and PL1 ($p < 0.001$), between PL1 and PL2 ($p = 0.023$), and between PL2 and T3 ($p < 0.001$) patients (Fig. 1).

The survival curves stratified by T and VPI status are shown in Figure 2*A*. Figure 2*B* shows the survival impact of VPI on T1a tumors. Although T1a tumors with VPI had a

TABLE 1. Patient Characteristics

	No. of Patients (%)				
	VPI Factor of T1/T2 Cases				
Characteristics	**PL0**	**PL1**	**PL2**	**T3**	**Total**
Age, yr					
Median (range)	67 (15–89)	68 (31–90)	68 (30–85)	69 (34–83)	67 (15–90)
Sex					
Men	2034 (56)	466 (64)	142 (64)	339 (77)	2981 (60)
Women	1572 (44)	261 (36)	77 (36)	104 (23)	2014 (40)
Surgery					
Lobectomy	3477 (96)	706 (97)	215 (98)	410 (93)	4808 (96)
Bilobectomy	95 (3)	12 (2)	3 (1)	12 (3)	122 (2)
Pneumonectomy	34 (1)	9 (1)	1 (1)	21 (5)	65 (1)
Histology					
Adenocarcinoma	2743 (76)	505 (70)	168 (77)	222 (50)	3638 (73)
Squamous cell carcinoma	660 (18)	168 (23)	37 (17)	163 (37)	1028 (21)
Adenosquamous carcinoma	55 (2)	14 (2)	2 (1)	13 (3)	84 (2)
Large-cell carcinoma	81 (2)	32 (4)	7 (3)	29 (7)	149 (3)
Others	67 (2)	8 (1)	5 (2)	16 (4)	96 (2)
Tumor diameter, cm					
<2	1558 (43)	199 (27)	40 (18)	29 (7)	1826 (37)
2.1–3	1125 (31)	215 (30)	72 (33)	71 (16)	1483 (30)
3.1–5	805 (22)	252 (35)	81 (37)	130 (29)	1268 (25)
5.1–7	118 (3)	61 (8)	26 (12)	72 (16)	277 (6)
≥7.1–	–	–	–	141 (32)	141 (3)
Total	3606	727	219	443	4995

VPI status was defined according to the 7th edition of the tumor, node, metastasis classification for lung and pleural tumors.
VPI, visceral pleural invasion

significantly poorer prognosis than T1a tumors without VPI ($p < 0.001$), there were no significant survival differences between T1a tumors with VPI and T1b tumors without VPI ($p = 0.083$) or T2a tumors without VPI ($p = 0.221$).

Figure 2*C* shows the survival impact of VPI on T1b tumors. Although T1b tumors with VPI had a significantly poorer prognosis than T1b tumors without VPI ($p = 0.001$), there were no significant survival differences between T1b

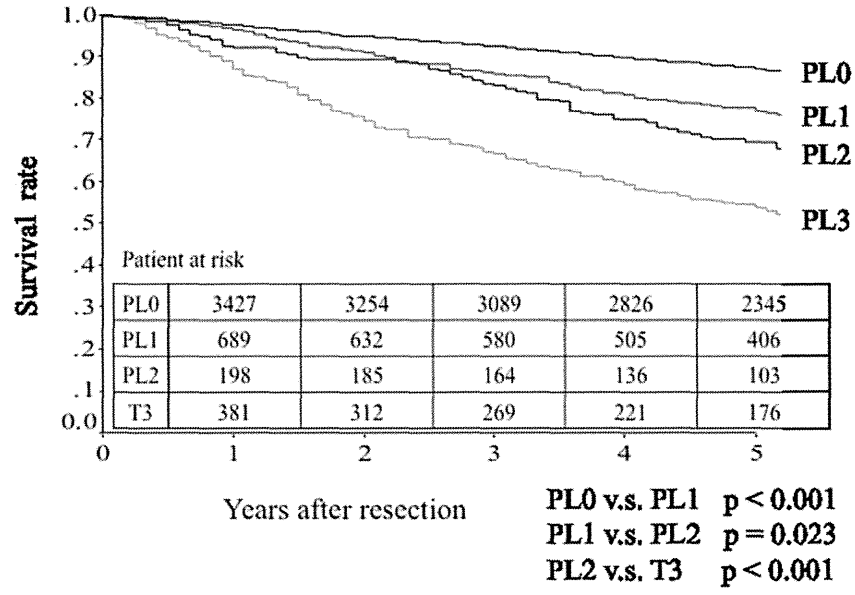

FIGURE 1. Overall survival curves of PL0, PL1, PL2, and T3 patients.

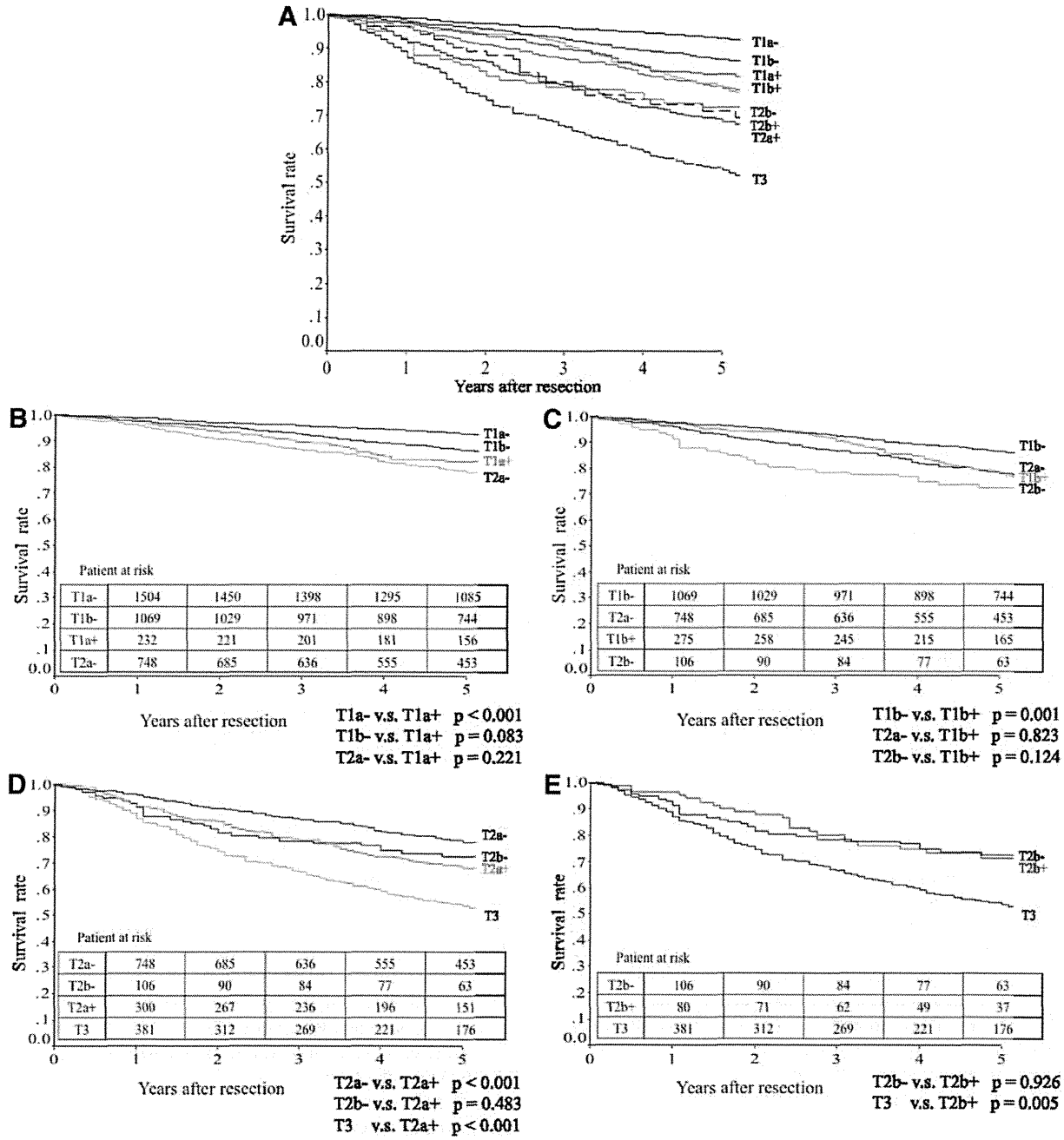

FIGURE 2. (*A*) Survival curves stratified by T stage and VPI status. (*B*) Survival curves of T1a/VPI–, T1b/VPI–, T2a/VPI–, and T1a/VPI+. (*C*) Survival curves of T1b/VPI–, T2a/VPI–, T2b/VPI–, and T1b/VPI+. (*D*) Survival curves of T2a/VPI–, T2b/VPI–, T2b/VPI–, and T2a/VPI+.(*E*) Survival curves of T2b/VPI–, T3, and T2b/VPI+.

tumors with VPI and T2a tumors without VPI ($p = 0.823$) or T2b tumors without VPI ($p = 0.124$).

Figure 2*D* shows the survival impact of VPI on T2a tumors. T2a tumors with VPI had a significantly poorer prognosis than T2a tumors without VPI ($p < 0.001$). There were no significant survival differences between T2a tumors with VPI and T2b tumors without VPI ($p = 0.483$). T2a tumors with VPI had a significantly better prognosis than T3 tumors ($p < 0.001$).

Figure 2*E* shows the survival impact of VPI on T2b tumors. There were no significant survival differences between T2b tumors with VPI and T2b tumors without VPI ($p = 0.926$). T2b tumors with VPI had a significantly better prognosis than T3 tumors ($p = 0.005$).

DISCUSSION

VPI is known to be a poor prognostic factor of NSCLC patients and is defined as a factor to upgrade T1a/T1b tumors to T2a in the 7th Edition of the TNM Classification for Lung and Pleural Tumours.[11,12,14] Travis et al.[13,17,18] recommend the use of elastic stains when invasion beyond the elastic layer is not clear on evaluation of HE sections. Although the Japan Lung Cancer Society also recommends using not only HE staining, but also elastic staining such as Victoria-blue van Gieson staining in VPI evaluation, the JJCLCR did not collect data regarding staining or how extensively VPI was evaluated in each participating institution. This is a major limitation of the present study.

In the present study, PL1 patients had a significantly poorer prognosis than PL0 patients, consistent with many previous reports.[1–10] PL2 patients had a significantly poorer prognosis than PL1 patients. The survival difference between PL1 and PL2 patients remains controversial. Kawase et al.[10] analyzed a cohort of more than 2700 patients, using the current VPI definition and elastic staining in all cases for VPI diagnosis, and reported no survival differences between PL1 and PL2 patients. Moreover, several other researchers have reported similar results.[2,6,9] In contrast, Sakakura et al.[4] reported significant differences in survival between PL1 and PL2 patients, but they did not describe whether or not they used elastic stains in diagnosing VPI status. In the data of the JJCLCR registry, it is not clear in what portion of the accumulated cases elastic staining was employed, and there remains some uncertainty regarding the determination of pleura invasion. Some PL0 patients might have been miscategorized as PL1 without the use of elastic staining, which may have led to the significant survival difference observed between PL1 and PL2 patients. To conclude whether or not a difference between PL1 and PL2 survival is valid, it is necessary to study more patients with VPI diagnoses made with the help of elastic staining.

To analyze the prognostic impact of VPI on T-status classification in the current cohort, we defined VPI to include PL1 and PL2 patients, as defined by the 7th edition of the TNM Classification for Lung and Pleural Tumours. T1a with VPI had a significantly poorer prognosis than T1a without VPI, but there were no significant survival differences between T1a with VPI and T1b without VPI, or between T1a with VPI and T2a without VPI. To summarize, T1a with VPI had prognosis similar to that of T1b/T2a without VPI, which suggests it is credible to upgrade T1a with VPI to T2a.

T1b with VPI had a significantly poorer prognosis than T1b without VPI, but there were no significant survival differences between T1b with VPI and T2a without VPI or between T1b with VPI and T2b without VPI. To summarize, T1b with VPI had a similar prognosis to T2a/T2b without VPI, which suggests it is reasonable to upgrade T1b with VPI to T2a, as described in the 7th edition of the TNM Classification for lung cancer.[11,12]

The most significant information of the present study is the outcome of T2a with VPI. T2a with VPI had a significantly poorer prognosis than T2a without VPI. There were no significant survival differences between T2a with VPI and T2b without VPI. T2a with VPI had a significantly better prognosis than T3. To summarize, T2a with VPI had a similar prognosis to T2b without VPI, which suggests T2a with VPI should be upgraded to T2b.

TABLE 2. T-Classification Comparison

Tumor Diameter, cm	VPI Ctatus	7th Edition T-Classification	Our Proposal
<2	–	T1a	T1a
<2	+	T2a	T2a (or T1b)
2.1–3	–	T1b	T1b
2.1–3	+	T2a	T2a
3.1–5	–	T2a	T2a
3.1–5	+	T2a	T2b
5.1–7	–	T2b	T2b
5.1–7	+	T2b	T2b

VPI– = PL0, VPI+ = PL1 or PL2.
VPI, visceral pleural invasion.

In the current cohort, there were no significant survival differences between T2b with VPI and T2b without VPI. T2b with VPI had a significantly better prognosis than T3. To summarize, T2b with VPI had a prognosis similar to that of T2b without VPI, which suggests there is no need to upgrade T2b with VPI. These suggestions are summarized in Table 2, and they include some differences from the conclusions of previous publications.[2,8,10]

A major limitation of the current study is that we do not know how thoroughly VPI was evaluated including elastic staining, in each participating institution. The differences observed may have been attributable to misdiagnoses of VPI status due to the lack of elastic staining use. However, the recommendation of the 7th edition of the TNM classification, that is, to upgrade T-classification according to VPI status, was determined on the basis of the results of some retrospective studies of small cohorts, in contrast to the large number cohort accumulated by the IASLC Lung Cancer Project. Moreover, the IASLC Lung Cancer Project also lacks detailed information on VPI status evaluation methodology. Therefore, we consider that a worldwide large-scale study that is limited to patients whose VPI status is diagnosed using elastic staining is necessary to determine the true impact on survival of pleural invasion and VPI.

In conclusion, in addition to the current TNM Classification recommendations—to upgrade tumors of 3 cm or less with VPI to T2a—tumors greater than 3 cm and 5 cm or less with VPI should be upgraded to T2b. However, more detailed further research is necessary for the next edition of the TNM classification for lung and pleural tumours, using a large-scale database with VPI status diagnosed using elastic staining.

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Forkhead box P3 regulatory T cells coexisting with cancer associated fibroblasts are correlated with a poor outcome in lung adenocarcinoma

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Recently, an association between tumor infiltrating Forkhead box P3 regulatory T cells (T_{reg}) and an unfavorable prognosis has been clinically shown in some cancers, but the mechanism of T_{reg} induction in the tumor microenvironment remains uncertain. The aims of the present study were to examine the relationship between T_{reg} and patient outcome and to investigate whether T_{reg} induction is influenced by the characteristics of cancer-associated fibroblasts (CAF) in lung adenocarcinoma. The numbers of T_{reg} in both the tumor stroma and the tumor nest were counted in 200 consecutive pathological stage I lung invasive adenocarcinoma specimens. To examine whether the characteristics of CAF influence T_{reg} induction, we selected and cultured CAF from low T_{reg} and high T_{reg} adenocarcinoma. The number of T_{reg} was much higher in the stroma than in the nest ($P < 0.01$). Patients with high T_{reg} had a significantly poorer prognosis than those with low T_{reg} (overall survival: $P = 0.03$; recurrence-free survival: $P = 0.02$; 5-year overall survival: 85.4% *vs* 93.0%). Compared with the CAF from low T_{reg} adenocarcinoma, culture supernatant of the CAF from high T_{reg} adenocarcinoma induced more T_{reg} ($P = 0.01$). Also, CAF from high T_{reg} adenocarcinoma expressed significantly higher mRNA levels of transforming growth factor-β ($P = 0.01$) and vascular endothelial growth factor ($P = 0.01$), both of which are involved in T_{reg} induction. Our studies suggest the possibility that CAF expressing immunoregulatory cytokines may induce T_{reg} in the stroma, creating a tumor-promoting microenvironment in lung adenocarcinoma that leads to a poor outcome. (*Cancer Sci* 2013; 104: 409–415)

Lung cancer is the most common cause of cancer-related death worldwide.[1] Surgery currently plays an important role in the treatment of clinical stage I–IIIA non-small lung cancer (NSCLC). Because of local recurrence and distant metastasis, however, patient outcome remains poor even after complete resection.[2] Effective therapies are needed for individual patients after surgery, and a new prognostic marker for the selection of patients with a high risk of cancer recurrence is required.

Both tumor cell characteristics and patients' immune responses have been shown to affect tumor development and metastasis.[3] Recent studies have shown that the accumulation of immunosuppressive lymphocytes, represented by regulatory T cells (T_{reg}) that suppress autoreactive T cells to maintain immunological self-tolerance and inhibit autoimmunity, is associated with advanced tumor growth and a poor outcome in several types of malignant tumors, including lung cancer.[4–8] Forkhead box P3 (Foxp3) is a member of the forkhead/winged-helix family of transcriptional factors that is critically involved in the development and function of T_{reg}.[9,10]

Cancer cells coexist with several stromal cell types that together create a cancer microenvironment. The main constituents of the stromal cell types are inflammatory cells, including lymphocytes and fibroblasts. Several recent reports have provided compelling experimental evidence indicating that the progression of tumors toward a malignant phenotype does not depend exclusively on the cell-autonomous properties of the cancer cells themselves; it is also deeply influenced by cancer associated fibroblasts (CAF).[11–13] Activated CAF contribute not only to inducing tumor progression, but also to creating the tumor microenvironment and inducing endothelial cells and other stromal cells via extracellular matrix proteins, proteases, cytokines and growth factors such as transforming growth factor (TGF)-β, human growth factor, vascular endothelial growth factor (VEGF), and fibroblast growth factor.[14] However, the correlation between tumor-infiltrating T_{reg} cells and CAF that express immunoregulatory cytokines has not been thoroughly investigated.

The aims of this study were to investigate the relationship between the T_{reg} number and the outcome of patients with p-stage I lung adenocarcinomas and to examine the possible correlation between T_{reg} induction in the tumor microenvironment and the characteristics of CAF.

Materials and Methods

Patients. The present study group comprises 200 consecutive patients with adenocarcinoma of the lung who underwent a complete resection at the National Cancer Center Hospital East, Kashiwa, Japan. All patients were diagnosed as having pathological stage I disease between January 2004 and December 2005, and all had a solitary lesion. Patients who had received preoperative chemotherapy or preoperative thoracic radiotherapy and whose tumor was diagnosed as a pure bronchioloalveolar carcinoma were excluded. All patients underwent a lobectomy or pneumonectomy for the resection of the primary lesion. We surveyed the patients at 3-month intervals for the first 2 years and at 6-month intervals thereafter.

The present research was approved by the Internal Review Board of the National Cancer Center Hospital East. The research consisted of a retrospective chart review in March 2012. No personally identifiable information was included.

Histopathological analysis and evaluation of clinicopathological factors. The available pathology slides from all 200 surgical specimens were coded, masked for identity, and then reviewed by two pathologists (T.K. and G.I.). The cases were reviewed according to the current (third) edition of the World Health

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