χ^2 test. Multivariate analyses were performed using the Cox proportional hazards regression model. All tests were two-sided, and p values of less than 0.05 were considered to indicate a statistically significant difference. Statview 5.0 software (SAS Institute Inc., Cary, NC) was used for statistical analyses. Data collection and analyses were approved and the need to obtain written informed consent from each patient was waived by the institutional review board of our institution.

RESULTS

The median follow-up for survivors was 5.1 years. Figure 1*A* and *B* show the OS and RFP curves of 316 patients with T1aN0M0 NSCLC and 216 patients with T1bN0M0 NSCLC. For those patients with T1aN0M0 NSCLC and those with T1bN0M0 NSCLC, the 5-year OS rates were 87.1% and 77.2% (p = 0.013), respectively, whereas the 5-year RFPs were 88.6% and 78.6% (p = 0.056), respectively.

Table 1 shows the 5-year OS proportions and RFPs according to the clinicopathological characteristics of the stage IA NSCLC patients. On univariate analysis, nine variables were found to be significantly associated ($p < 0.05$) with poorer OS: older age, male sex, smoking history, T1b, poorly differentiated carcinoma, blood-vessel invasion, lymph-vessel invasion, nonadenocarcinoma, and type of surgery (bilobectomy or pneumonectomy). For RFP, five variables (male sex, poorly differentiated carcinoma, blood-vessel invasion, lymph-vessel invasion, and nonadenocarcinoma) were identified as statistically significant factors on univariate analysis.

A multivariate Cox proportional hazards model demonstrated that older age (hazard ratio [HR] = 1.936; $p < 0.001$), male sex (HR = 2.096; p = 0.005), tumor size (HR = 1.501; p = 0.045), poorly differentiated carcinoma (HR = 1.632; p = 0.028), lymph-vessel invasion (HR = 1.579; p = 0.042), and nonadenocarcinoma (HR = 1.704; p = 0.016) were statistically significant predictors of OS (Table 2). Poorly differentiated carcinoma (HR = 1.925; p = 0.006), blood-vessel invasion (HR = 1.712; p = 0.020), and lymph-vessel invasion (HR = 1.751; p = 0.017) were identified as statistically significant predictors of cancer recurrence (Table 3). Figures 2*A*, *B*, and *C* show the RFP curves of patients with stage IA NSCLC according to tumor differentiation, blood-vessel invasion, and lymph-vessel invasion, respectively. Table 4 shows the results of 5-year RFP of patients in each T subclassification (T1a and T1b) according to these significant predictors of cancer recurrence.

Subgroup analysis with a combination of these recurrence predictive factors in the patients with stage IA NSCLC revealed 5-year RFPs of 91.3%, 79.5%, and 62.9% for patients with no risk factor, poorly differentiated carcinoma or vascular invasion (blood-vessel invasion or lymph-vessel

TABLE 1. Patient Characteristics and Univariate Analysis of Survival and Recurrence

Variable	No. of Patients	5-Yr OSP (%)	p Value	5-Yr RFP (%)	p Value
Age (yrs: median 64)					
< 64	279	88.9		84.2	
≥ 64	253	76.6	< 0.001	85.3	0.946
Sex					
Male	290	77.7		81.4	
Female	242	89.6	< 0.001	88.4	0.009
Smoking status					
Ever smoker	279	81.5		82.6	
Never smoker	253	84.9	0.039	86.8	0.102
CEA (ng/ml: NUL of 5)					
< 5	447	83.7		85.2	
≥ 5	59	75.9	0.108	77.2	0.212
Tumor size					
T1a (≤ 2.0 cm)	316	87.1		88.6	
T1b (≥ 2.1 cm)	216	77.2	0.013	78.6	0.056
Differentiation					
Well or moderate	425	86.4		87.7	
Poor	96	71.4	< 0.001	71.8	< 0.001
Blood-vessel invasion					
Absent	402	86.2		88.1	
Present	116	72.1	0.002	71.3	< 0.001
Lymph-vessel invasion					
Absent	392	85.4		87.1	
Present	122	76.4	0.003	76.1	0.001
Histology					
Adenocarcinoma	439	86.6		86.6	
Nonadenocarcinoma	93	66.3	< 0.001	74.3	< 0.001
Tumor laterality					
Right	357	82.9		84.3	
Left	175	83.6	0.685	85.4	0.732
Type of surgery					
Single-lobe lobectomy	510	84.0		84.5	
More extensive resection (more than bilobectomy)	22	66.7	0.046	88.7	0.946

OSP, overall survival proportion; RFP, recurrence-free proportion; NUL, normal upper limit; CEA, preoperative serum carcinoembryonic antigen level.

TABLE 2. Multivariate Cox Proportional Hazards Regression Analysis of Overall Survival

Variable	Risk Factors	Hazard Ratio	95% Confidence Interval	p Value
Age	≥ 64	1.936	1.314–2.852	< 0.001
Sex	Male	2.096	1.251–3.510	0.005
Smoking status	Ever smoker	1.219	0.781–1.901	0.383
Tumor size	T1b (≥ 2.1 cm)	1.501	1.009–2.233	0.045
Differentiation	Poor	1.632	1.054–2.527	0.028
Blood-vessel invasion	Present	1.169	0.749–1.827	0.492
Lymph-vessel invasion	Present	1.579	1.017–2.449	0.042
Histology	Nonadenocarcinoma	1.704	1.103–2.632	0.016
Type of surgery	More extensive resection (more than bilobectomy)	1.981	0.984–3.984	0.055

TABLE 3. Multivariate Cox Proportional Hazards Regression Analysis of Cancer Recurrence

Variable	Risk Factors	Hazard Ratio	95% Confidence Interval	*p* Value
Sex	Male	1.171	0.747–1.834	0.492
Differentiation	Poor	1.925	1.210–3.063	0.006
Blood-vessel invasion	Present	1.712	1.088–2.694	0.020
Lymph-vessel invasion	Present	1.751	1.103–2.779	0.017
Histology	Nonadenocarcinoma	1.615	0.994–2.623	0.053

invasion), and both poorly differentiated carcinoma and vascular invasion, respectively (Fig. 3*A*). The differences in RFP were statistically significant between patients without any risk factors (A group) and those with poorly differentiated carcinoma or vessel invasion (B group) ($p < 0.001$). The 5-year RFP of patients with both poorly differentiated carcinoma and vascular invasion (C group) tended to be unfavorable compared with that of patients in the B group, but the difference was not statistically significant ($p = 0.068$). In patients with T1a, the 5-year RFP of patients without any risk factors (A group) was statistically different from that of patients with poorly differentiated carcinoma or vessel invasion (B group) (92.0% versus 83.7% in A and B, respectively; $p = 0.002$), whereas

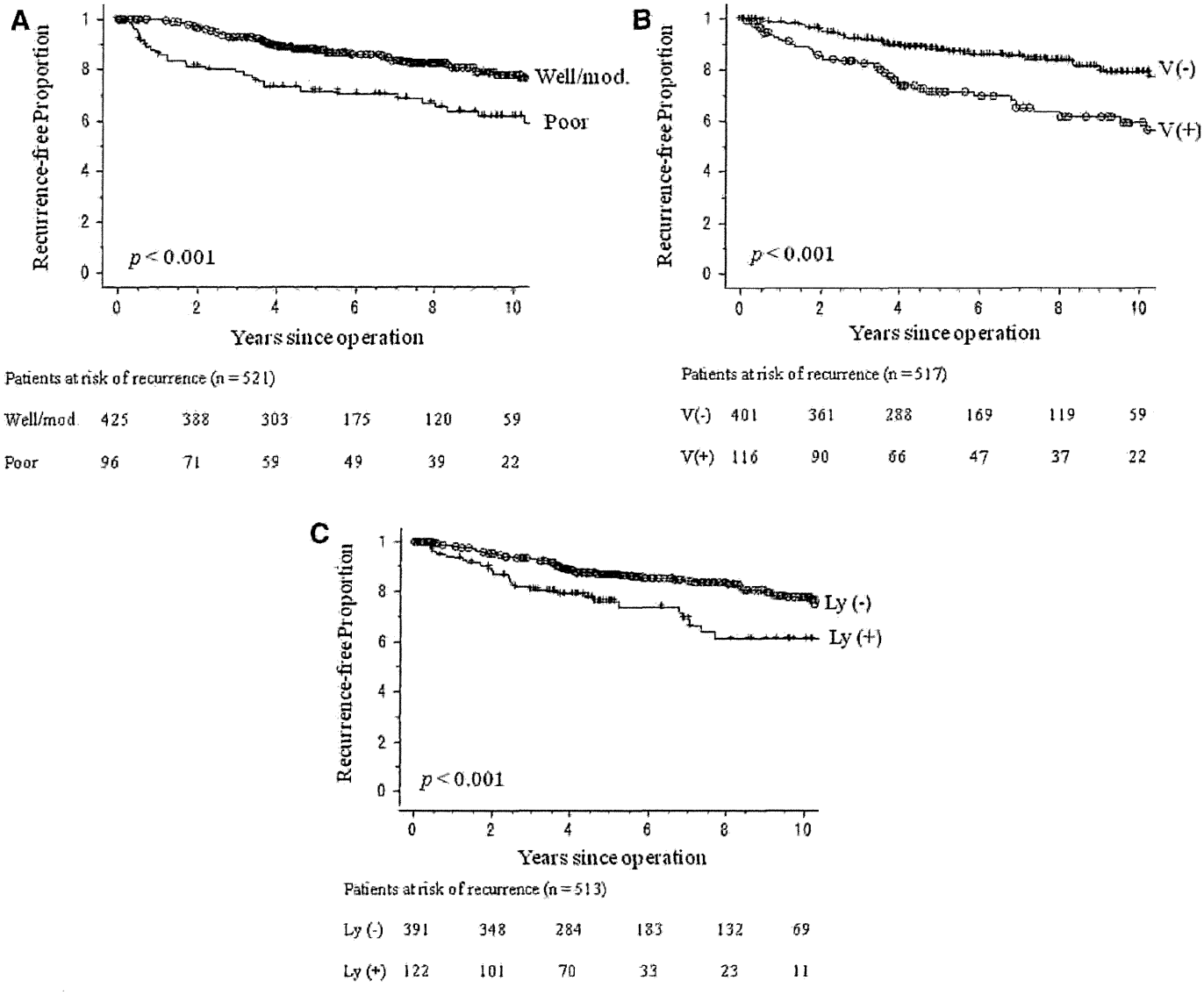

FIGURE 2. *A*, Recurrence-free proportion curves according to tumor differentiation. *B*, Recurrence-free proportion curves according to blood-vessel invasion. *C*, Recurrence-free proportion curves according to lymph-vessel invasion.

TABLE 4. 5-Year Recurrence-Free Proportion for Each T Subclassification According to Histological Grade and Vascular-Invasion Status

T-Factor category	No. of Patients	5-Yr RFP (%)	*p* Value
T1a (≤ 2.0 cm)			
Well/mod.	249	90.3	
Poor	60	83.8	0.126
T1b (≥ 2.1 cm)			
Well/mod.	176	83.7	
Poor	36	51.3	< 0.001
T1a (≤ 2.0 cm)			
BVI (−)	265	90.2	
BVI (+)	44	77.5	0.005
T1b (≥ 2.1 cm)			
BVI (−)	137	83.8	
BVI (+)	72	67.0	0.011
T1a (≤ 2.0 cm)			
LVI (−)	252	90.2	
LVI (+)	54	79.4	0.003
T1b (≥ 2.1 cm)			
LVI(−)	140	81.4	
LVI (+)	68	73.2	0.181

RFP, recurrence-free proportion; Well/mod., well- or moderately differentiated carcinoma; Poor, poorly differentiated carcinoma; BVI, blood-vessel invasion; LVI, lymph-vessel invasion.

no significant difference was shown between patients in the B group and those with both poorly differentiated carcinoma and vascular invasion (C group; 79.4% at 5-year RFP for C group; $p = 0.812$) (Fig. 3*B*). The RFP curves for T1b patients of the A, B, and C groups were shown in Fig. 3*C*. The differences in recurrence were statistically significant between A and B (89.6% versus 75.1% at 5-year RFP in A and B, respectively; $p = 0.006$), B and C (43.3% at 5-year RFP for the C group; $p = 0.002$).

We tested for a correlation between histological grade or vascular-invasion status and clinicopathological variables in stage IA patients. A comparison of variables between well- or moderately differentiated carcinoma and poorly differentiated carcinoma groups showed that a statistically significant difference in the prevalence of poorly differentiated carcinoma was seen in patients of male sex ($p < 0.001$), those who were smokers ($p < 0.001$) those in whom vascular invasion was present ($p < 0.001$), and those who had nonadenocarcinoma histology ($p < 0.001$). Vascular invasion was significantly associated with male sex ($p = 0.035$), smoking ($p = 0.001$), T1b ($p < 0.001$), and poorly differentiated carcinoma ($p < 0.001$) (data not shown).

Table 5 shows the number of patients with recurrence and their initial recurrence pattern according to histological grade and vascular-invasion status. The proportion of patients who developed distant metastases was higher in these recurrence predictive factor positive populations than in the negative populations (histological grade; $p = 0.048$, vascular invasion; $p = 0.024$).

DISCUSSION

We set out to identify the clinicopathological factors that affect overall prognosis and cancer recurrence of stage IA NSCLC. Curative surgical resection is the most effective therapy for patients with stage IA NSCLC. However, a considerable number of patients develop recurrence, which results in cancer death. Previous studies have reported the following factors to be associated with a poor prognosis in patients with stage IA NSCLC: tumor size,[5] preoperative serum CEA level,[7] lymph-vessel invasion,[18] blood-vessel invasion,[7,13–15,17] and histological grade.[14,17,19] In addition, according to the Surveillance, Epidemiology, and End Result Program database, age, sex, and extent of resection are also important prognostic factors.[22] However, prognostic factors such as age and sex do not accurately predict or explain recurrence in patients with stage IA NSCLC. Therefore, we focused on the risk factors for recurrence and unfavorable OS in the present study. When describing the survival experience of a group of patients, the OS parameter is typically used. However, OS is affected by death resulting from causes other than lung cancer itself, including complications and comorbidities, and is considered to be affected by treatment after relapse. For example, epidermal growth factor receptor tyrosine kinase inhibitors are highly effective against mutated epidermal growth factor receptor recurrent NSCLC patients, suggesting potential improvements in postoperative survival regardless of surgery effect. Therefore, in evaluating pure surgical impact on the natural history of early-stage NSCLC, we consider that RFP may be a better prognostic indicator than OS. On multivariate analyses, we identified five independently significant predictors for poor prognosis: older age (HR = 1.936), male sex (HR = 2.096), tumor size (HR = 1.501), poorly differentiated carcinoma (HR = 1.632), lymph-vessel invasion (HR = 1.579), and nonadenocarcinoma (HR = 1.704); we also identified three predictors of recurrence: poorly differentiated carcinoma (HR = 1.925), blood-vessel invasion (HR = 1.712), and lymph-vessel invasion (HR = 1.751). The present study showed that independent predictive factors of poor survival were slightly different from predictive factors of recurrence.

Several authors reported that patients with poor differentiated carcinomas after resection had a higher risk of recurrence and death.[14,23,24] Although the histological grading system may provide useful information in defining the aggressiveness of tumors and has a significant impact on the survival of patients,[19] the four-tiered system of grading (well-differentiated, moderately differentiated, poorly differentiated, and undifferentiated carcinomas) for lung cancer is assumed to lack objectivity, because no original criteria have been developed for standardizing lung cancer histology. However, the current result indicates that poor differentiation contributes to unfavorable clinical outcome, suggesting that this factor may be a useful indicator of a need for postoperative adjuvant chemotherapy in patients with stage IA NSCLC. Consistent grading criteria need to be established for reproducible assessment.

Blood-vessel invasion is considered to be a fundamental step in hematogenous metastasis. The presence of blood-vessel invasion was previously found to be a strong

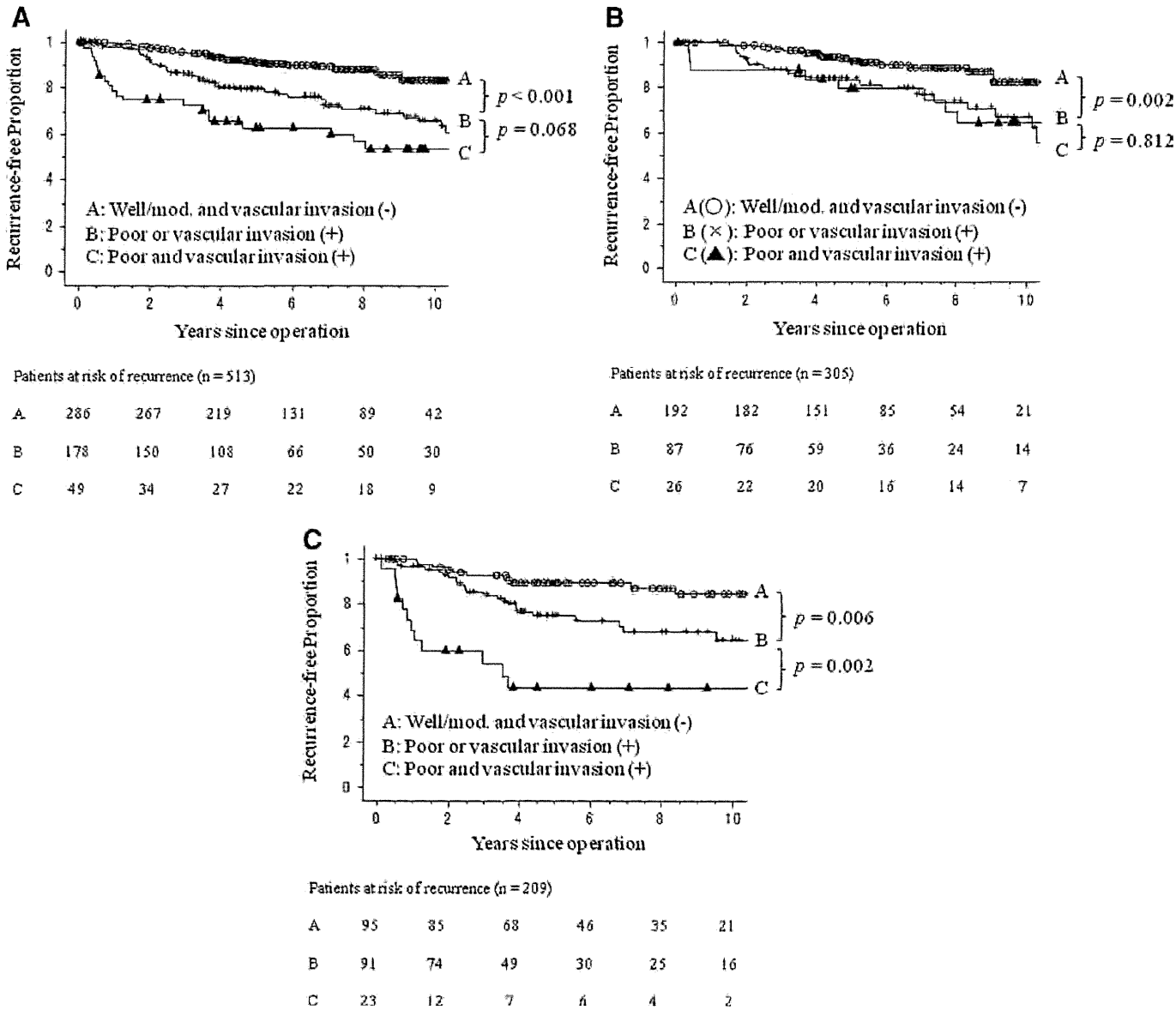

FIGURE 3. *A*, Recurrence-free proportion curves for all stage IA; *B*, T1a ; and *C*, T1b patients with well- or moderately differentiated carcinoma and no vascular invasion (curve A), poorly differentiated carcinoma or vascular invasion (curve B), and both poorly differentiated carcinoma and vascular-invasion (curve C).

independent unfavorable prognostic factor, and vascular invasion should be considered for inclusion in the staging criteria and indications for adjuvant chemotherapy.[10,11,13] Fujisawa et al.[25] demonstrated that blood-vessel invasion is a very important prognostic factor in resected NSCLCs with intrapulmonary metastasis, and may correlate with the anatomical aspect of pulmonary metastasis. The current study also suggests that the presence of blood-vessel invasion is a significant risk factor for recurrence in stage IA NSCLC patients.

To identify blood-vessel invasion more accurately, we used hematoxylin and eosin and EvG stains to visualize elastic fibers in all cases. We recommend the routine use of elastic stains in the pathological evaluation of lung cancer, not only for the determination of visceral pleural invasion but also for the determination of blood-vessel invasion, particularly in patients with stage IA NSCLC.

Lymph-vessel invasion has been reported to be an independent indicator of cancer invasiveness and poor prognosis in most studies that included this factor in their analyses.[9,18,26,27] The present study shows that as it is for histological grade, lymph-vessel invasion was a significant predictor of both poor prognosis and cancer recurrence, surpassing tumor size in pathologic stage IA NSCLC.

Recent randomized controlled trials have demonstrated the usefulness of postoperative adjuvant chemotherapy in stage IB to IIIA NSCLC patients who have undergone complete resections.[28–30] Although surgery alone remains the

TABLE 5. Initial Observed Cancer Recurrence Patterns of Patients According to Histological Grade and Vascular-Invasion Status

Initial Recurrence Pattern	Tumor Differentiation			Vascular Invasion		
	Well/ mod.	Poor	*p* Value	Absent	Present	*p* Value
Overall (%)	425 (82)	96 (18)		340 (65)	180 (35)	
Patients with recurrence (%)	64 (15)	35 (36)		46 (14)	53 (29)	
Local recurrence only	24 (38)	9 (25)	0.048	19 (41)	14 (26)	0.025
Distant recurrence	39 (62)	27 (75)		27 (59)	39 (74)	

Well/mod., well- or moderately differentiated carcinoma; Poor, poorly differentiated carcinoma.

standard treatment for patients with stage IA NSCLC, larger studies on resected cases comparing uracil-tegafur adjuvant chemotherapy versus observation showed that uracil-tegafurimproved survival for patients with stage I adenocarcinoma, and also showed a clear survival benefit in the T1-disease subgroup of patients with a tumor of diameter more than 2 cm.[31,32] However, tumor size might not be the only factor found to have a benefit on adjuvant chemotherapy after complete resection of stage IA NSCLC. In the present study, when we divided the study population into A (patients without any risk factors), B (those with either poorly differentiated carcinoma or vascular invasion), and C (those with both poorly differentiated carcinoma and vascular invasion) groups, the 5-year RFP of all stage IA patients were 91.3%, 79.5%, and 62.9%, respectively. In particular, the subgroup analysis of patients with stage IA disease stratified by tumor size showed a 5-year RFP of 43.3% for the T1b C group. These results indicated high-risk small-tumor N0 patients, identified by factors other than tumor size, such as tumor differentiation and vascular invasion, may be good candidates for adjuvant chemotherapy.

This study has limitations and biases that should be mentioned. As a retrospective single-institute study, patient-selection bias and time-trend bias regarding the diagnosis for cancer recurrence might be inevitable compared with multi-institutional prospective study. Moreover, the definition of an ipsilateral lung metastasis as a local recurrence also generated inherent bias while allowing the differentiation of a new primary lung cancer from a recurrent NSCLC.

The anatomical extent of disease, as described by the TNM for lung and pleural tumors, remains the most powerful prognostic instrument in NSCLC. A challenge for the future will be to integrate the TNM with specific pathological factors, such as vascular-invasion status or tumor differentiation, to create a composite prognostic index for NSCLC.

CONCLUSION

Even though most patients comprised an early-staging subset, those with stage IA NSCLC comprised a heterogeneous group with different prognoses and risk of cancer recurrence. The current study demonstrates that vascular-invasion status and tumor differentiation were far more powerful recurrence predictive factors than tumor size, and this information can be useful for the selection of the appropriate therapeutic strategy, including adjuvant chemotherapy, which can be tailored to the individual patient's risk of developing recurrence.

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REFERENCES

1. Goya T, Asamura H, Yoshimura H, et al.; Japanese Joint Committee of Lung Cancer Registry. Prognosis of 6644 resected non-small cell lung cancers in Japan: a Japanese lung cancer registry study. *Lung Cancer* 2005;50:227–234.
2. Asamura H, Goya T, Koshiishi Y, et al.; Japanese Joint Committee of Lung Cancer Registry. A Japanese Lung Cancer Registry study: prognosis of 13,010 resected lung cancers. *J Thorac Oncol* 2008;3:46–52.
3. Sawabata N, Miyaoka E, Asamura H, et al.; Japanese Joint Committee for Lung Cancer Registration. Japanese lung cancer registry study of 11,663 surgical cases in 2004: demographic and prognosis changes over decade. *J Thorac Oncol* 2011;6:1229–1235.
4. Rami-Porta R, Ball D, Crowley J, et al.; International Staging Committee; Cancer Research and Biostatistics; Observers to the Committee; Participating Institutions. The IASLC Lung Cancer Staging Project: proposals for the revision of the T descriptors in the forthcoming (seventh) edition of the TNM classification for lung cancer. *J Thorac Oncol* 2007;2:593–602.
5. Agarwal M, Brahmanday G, Chmielewski GW, Welsh RJ, Ravikrishnan KP. Age, tumor size, type of surgery, and gender predict survival in early stage (stage I and II) non-small cell lung cancer after surgical resection. *Lung Cancer* 2010;68:398–402.
6. Kawai H, Tada A, Kawahara M, et al.; Japan National Hospital Study Group for Lung Cancer. Smoking history before surgery and prognosis in patients with stage IA non-small-cell lung cancer–a multicenter study. *Lung Cancer* 2005;49:63–70.
7. Okada M, Sakamoto T, Nishio W, Uchino K, Tsubota N. Characteristics and prognosis of patients after resection of nonsmall cell lung carcinoma measuring 2 cm or less in greatest dimension. *Cancer* 2003;98:535–541.
8. Ruffini E, Asioli S, Filosso PL, et al. Significance of the presence of microscopic vascular invasion after complete resection of Stage I-II pT1-T2N0 non-small cell lung cancer and its relation with T-Size categories: did the 2009 7th edition of the TNM staging system miss something? *J Thorac Oncol* 2011;6:319–326.
9. Bréchot JM, Chevret S, Charpentier MC, et al. Blood vessel and lymphatic vessel invasion in resected nonsmall cell lung carcinoma. Correlation with TNM stage and disease free and overall survival. *Cancer* 1996;78:2111–2118.
10. Tsuchiya T, Akamine S, Muraoka M, et al. Stage IA non-small cell lung cancer: vessel invasion is a poor prognostic factor and a new target of adjuvant chemotherapy. *Lung Cancer* 2007;56:341–348.
11. Miyoshi K, Moriyama S, Kunitomo T, Nawa S. Prognostic impact of intratumoral vessel invasion in completely resected pathologic stage I non-small cell lung cancer. *J Thorac Cardiovasc Surg* 2009;137:429–434.
12. Shimada Y, Ishii G, Hishida T, Yoshida J, Nishimura M, Nagai K. Extratumoral vascular invasion is a significant prognostic indicator and a predicting factor of distant metastasis in non-small cell lung cancer. *J Thorac Oncol* 2010;5:970–975.
13. Maeda R, Yoshida J, Ishii G, Hishida T, Nishimura M, Nagai K. Prognostic impact of intratumoral vascular invasion in non-small cell lung cancer patients. *Thorax* 2010;65:1092–1098.
14. Ichinose Y, Yano T, Asoh H, Yokoyama H, Yoshino I, Katsuda Y. Prognostic factors obtained by a pathologic examination in completely

resected non-small-cell lung cancer. An analysis in each pathologic stage. *J Thorac Cardiovasc Surg* 1995;110:601–605.
15. Thomas P, Doddoli C, Thirion X, et al. Stage I non-small cell lung cancer: a pragmatic approach to prognosis after complete resection. *Ann Thorac Surg* 2002;73:1065–1070.
16. Maeda R, Yoshida J, Ishii G, Hishida T, Nishimura M, Nagai K. Poor prognostic factors in patients with stage IB non-small cell lung cancer according to the seventh edition TNM classification. *Chest* 2011;139:855–861.
17. Maeda R, Yoshida J, Ishii G, et al. Long-term survival and risk factors for recurrence in stage I non-small cell lung cancer patients with tumors up to 3 cm in maximum dimension. *Chest* 2010;138:357–362.
18. Harada M, Hato T, Horio H. Intratumoral lymphatic vessel involvement is an invasive indicator of completely resected pathologic stage I non-small cell lung cancer. *J Thorac Oncol* 2011;6:48–54.
19. Kobayashi N, Toyooka S, Soh J, et al. Risk factors for recurrence and unfavorable prognosis in patients with stage I non-small cell lung cancer and a tumor diameter of 20 mm or less. *J Thorac Oncol* 2007;2:808–812.
20. Leslie Sobin MG CW (Ed). TNM Classification of Malignant Tumors, 7th Ed. Geneva, UICC International Union Against Cancer, 2009, pp 136–146.
21. William D, Travis EB HKM-H, Curtis C. Harris (Ed). Pathology and Genetics of Tumors of the Lung, Pleura, Thymus and Heart. Lyon, World Health Organization Classification of Tumours, 2004, pp 9–124.
22. Chang MY, Mentzer SJ, Colson YL, et al. Factors predicting poor survival after resection of stage IA non-small cell lung cancer. *J Thorac Cardiovasc Surg* 2007;134:850–856.
23. Sun Z, Aubry MC, Deschamps C, et al. Histologic grade is an independent prognostic factor for survival in non-small cell lung cancer: an analysis of 5018 hospital- and 712 population-based cases. *J Thorac Cardiovasc Surg* 2006;131:1014–1020.
24. Chung CK, Zaino R, Stryker JA, O'Neill M Jr, DeMuth WE Jr. Carcinoma of the lung: evaluation of histological grade and factors influencing prognosis. *Ann Thorac Surg* 1982;33:599–604.
25. Fujisawa T, Yamaguchi Y, Saitoh Y, Hiroshima K, Ohwada H. Blood and lymphatic vessel invasion as prognostic factors for patients with primary resected nonsmall cell carcinoma of the lung with intrapulmonary metastases. *Cancer* 1995;76:2464–2470.
26. Shimizu K, Yoshida J, Nagai K, et al. Visceral pleural invasion is an invasive and aggressive indicator of non-small cell lung cancer. *J Thorac Cardiovasc Surg* 2005;130:160–165.
27. Saijo T, Ishii G, Ochiai A, et al. Evaluation of extratumoral lymphatic permeation in non-small cell lung cancer as a means of predicting outcome. *Lung Cancer* 2007;55:61–66.
28. Winton T, Livingston R, Johnson D, et al.; National Cancer Institute of Canada Clinical Trials Group; National Cancer Institute of the United States Intergroup JBR.10 Trial Investigators; . Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med* 2005;352:2589–2597.
29. Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J; International Adjuvant Lung Cancer Trial Collaborative Group. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med* 2004;350:351–360.
30. Douillard JY, Rosell R, De Lena M, et al. Adjuvant vinorelbine plus cisplatin versus observation in patients with completely resected stage IB-IIIA non-small-cell lung cancer (Adjuvant Navelbine International Trialist Association [ANITA]): a randomised controlled trial. *Lancet Oncol* 2006;7:719–727.
31. Kato H, Ichinose Y, Ohta M, et al.; Japan Lung Cancer Research Group on Postsurgical Adjuvant Chemotherapy. A randomized trial of adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung. *N Engl J Med* 2004;350:1713–1721.
32. Hamada C, Tsuboi M, Ohta M, et al. Effect of postoperative adjuvant chemotherapy with tegafur-uracil on survival in patients with stage IA non-small cell lung cancer: an exploratory analysis from a meta-analysis of six randomized controlled trials. *J Thorac Oncol* 2009;4:1511–1516.

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Retrospective Analysis of Nodal Spread Patterns According to Tumor Location in Pathological N2 Non-small Cell Lung Cancer

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Abstract
Background The purpose of the present study was to determine the nodal spread patterns of pN2 non-small cell lung cancer (NSCLC) according to tumor location, and to attempt to evaluate the possible indications of selective lymph node dissection (SLND).
Methods We retrospectively analyzed nodal spread patterns in 207 patients with NSCLC of less than 5 cm with N2 involvement.
Results The tumor location was right upper lobe (RUL) in 79, middle lobe in 12, right lower lobe (RLL) in 40, left upper division (LUD) in 41, lingular division in 11, and left lower lobe (LLL) in 24. Both RUL and LUD tumors showed a higher incidence of upper mediastinal (UM) involvement (96 and 100 %, respectively) and a lower incidence of subcarinal involvement (15 and 10 %, respectively) than lower lobe tumors (UM; RLL 60 %, LLL 42 %; subcarinal: RLL 60 %, LLL 46 %, respectively). Among the patients with 24 right UM-positive RLL and 10 left UM-positive LLL tumors, 2 showed negative hilar, subcarinal, and lower mediastinal involvement, and cT1, suggesting that UM dissection may be unnecessary in lower lobe tumors with no metastasis to hilar, subcarinal, and lower mediastinal nodes on frozen sections according to the preoperative T status. Among the patients with 12 subcarinal-positive RUL and 4 subcarinal-positive LUD tumors, one showed negative hilar or UM involvement, suggesting that subcarinal dissection may be unnecessary in RUL or LUD tumors with no metastasis to hilar and UM nodes on frozen sections.
Conclusions The present study appears to provide one of the supportive results regarding the treatment strategies for tumor location-specific SLND.

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Introduction

Lobectomy with systematic mediastinal lymph node dissection (LND) has been considered the standard of care for resectable non-small cell lung cancer (NSCLC). Lymph node dissection was first reported by Cahan in 1960 [1] and is known to enhance staging accuracy by increasing lymph node harvesting and improving the identification of occult N2 disease. In contrast, other investigators claim that LND can potentially increased postoperative morbidity or may require longer operative time [2–5]. Some randomized controlled trials addressing the survival benefit of LND and mediastinal lymph node sampling showed no difference in survival outcome between patients undergoing LND and those undergoing lymph node sampling [3, 6, 7]. Whether or not patient outcome is improved by LND remains controversial.

At present, early lung cancers are more frequently encountered because of the widespread use of high-resolution computed tomography (CT) in routine practice and cancer screening [8, 9]. Therefore, the extent of LND should be tailored to each patient. Selective lymph node dissection (SLND) based on the tumor location-specific lymphatic pathway should be undertaken especially for patients with no apparent lymph node metastasis or with impaired pulmonary function, or for elderly patients. In the present study, we retrospectively reviewed the prevalence of lymph node involvement in each mediastinal region in

patients with N2 NSCLC according to the location of the primary tumor, and we attempted to evaluate the possible indications for SLND.

Patients and methods

Patients

From January 1990 to December 2007, a total of 2,195 patients underwent radical surgical resection of at least a lobectomy and systematic LND for NSCLC at our hospital. Of these 2,195 patients, we retrospectively analyzed lymph node spread patterns and outcome in 207 patients with NSCLC of less than 5 cm with N2 involvement. We excluded patients who had received preoperative treatment, including chemotherapy or chemoradiotherapy, those who had undergone only biopsy and SLND, and those who had low-grade malignant tumors. We also excluded patients with tumors spreading across lobar fissures and invading multiple lobes.

Preoperative evaluation included physical examination, chest radiography, computed tomography (CT) of the chest and abdomen, magnetic resonance imaging of the brain, bone scintigraphy, and blood examination. We determined that a large lymph node over 10 mm in the shortest axis was positive for metastasis on CT scans. Positron-emission tomography (PET) scan (recently integrated PET-CT scan) was not routinely used for staging resectable tumors during the study period. In recent years, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was sometimes performed for the patients having suspected multiple N2 lymph node metastases, but it was not routinely used. Similarly, mediastinoscopic biopsy was not routinely performed. Patients with N2 lymph node positively diagnosed by EBUS-TBNA or mediastinoscopic biopsy were excluded from the group of operative indication candidates.

The stage of disease was determined according to the 2009 7th Edition of the TNM Classification for Lung and Pleural Tumors [10]. The institutional review board of our institution approved the data collection and analyses and waived the need to obtain written informed consent from each patient.

Operation

During thoracotomy, lymph nodes in the ipsilateral thoracic cavity were completely resected. Systematic nodal dissection, including the superior to inferior mediastinum, was then performed after pulmonary resection. In cases of left thoracotomy, upper mediastinal dissection indicated aortic and tracheobronchial node dissection. If intraoperative findings showed that hilar or mediastinal lymph nodes were highly suspicious for metastatic disease, the resected lymph node specimens were immediately examined pathologically in frozen sections. Whether or not the presence or absence of lymph node metastasis was judged by intraoperative diagnosis, systematic LND was performed in the present study patients. Mediastinal metastases were considered to be skip metastases if any of the N2 nodes, but not the N1 nodes, were involved.

Mediastinal lymph node stations were grouped into the "zones" proposed by the International Association for the Study of Lung Cancer (IASLC) lung cancer staging project [11]. We also reviewed the correlation between nodal zone spread pattern and tumor location. We classified lymph node stations into the following six zones: the right upper (RU) and left upper (LU) zones, each including #2R, #3a, #3p, and #4R nodes; the subcarinal (SC) zone, including #7 nodes; the right lower (RL) and left lower (LL) zones, each including #8 and #9 nodes; and the aortic-pulmonary (AP) zone, including #5, and #6 nodes.

Statistical analysis

Overall survival time was measured from the date of surgery to the date of death from any cause or the date on which the patient was last known to be alive. Survival curves were plotted according to the Kaplan–Meier method and compared with the log-rank test. Two-category comparison was performed by the Pearson χ^2 test and Fisher's exact test for quantitative data. All tests were two-sided, and p values of <0.05 were considered to indicate statistically significant differences. We used StatView 5.0 (SAS Institute Inc., Cary, NC) for the statistical analysis.

Results

Patient characteristics are summarized in Table 1. Of the 207 patients with NSCLC of less than 5 cm with N2 involvement, 55 (27 %) had skip metastasis, and 97 (47 %) had both hilar and the remaining 55 patients had metastatic segmental lymph nodes or subsegmenta lymph nodes with mediastinal lymph nodes metastasis. In addition, 74 (36 %) were diagnosed with cN2 disease by the chest CT. Lymph node spread patterns according to primary tumor location are presented in Fig. 1. The most common site of involvement for tumors of the right upper lobe (RUL; $n = 79$) was the RU zone ($n = 76$; Fig. 1a). Right upper lobe tumors showed a significantly higher incidence of RU zone metastasis than right lower lobe (RLL) tumors (96 vs. 60 %, $p < 0.001$: Fig. 1a, b). In contrast, when RU zone metastasis was present, RLL tumors showed a significantly higher incidence of simultaneous metastasis to the SC or RL zone

Table 1 Patient characteristics ($n = 207$)

	n	(%)
Overall	207	(100)
Sex		
Male	134	(65)
Female	73	(35)
Histologic type		
Adenocarcinoma	149	(72)
Squamous cell carcinoma	41	(20)
Others	17	(8)
Tumor size (cm)		
2.0	38	(18)
2.1–3.0	55	(27)
3.1–5.0	114	(55)
p-T status		
pT1	47	(23)
pT2	129	(62)
pT3	18	(9)
pT4	13	(6)
Hilar lymph node metastasis		
Present	97	(47)
Absent	110	(53)
Skip metastasis		
Present	55	(27)
Absent	152	(73)
Tumor location		
Right upper lobe	79	(38)
Right middle lobe	12	(6)
Right lower lobe	40	(19)
Left upper division	41	(20)
Left lingular division	11	(5)
Left lower lobe	24	(12)
Procedure		
Pneumonectomy	15	(7)
Bilobectomy	19	(9)
Lobectomy	173	(84)

than RUL tumors (28 vs. 11 %, $p = 0.026$: Fig. 1a, b). The incidence of skip metastasis to only the RU zone was statistically lower among patients with RLL tumors than among those with RUL tumors (8 vs. 30 %, $p = 0.005$: Fig. 1a, b). Right upper lobe tumors showed a significantly lower incidence of SC zone metastasis than RLL tumors (15 vs. 60 %, $p < 0.001$: Fig. 1c, d). Most RUL tumors with SC zone metastasis showed simultaneous metastasis to the RU zone or hilar lymph nodes, and only one patient showed skip metastasis to the SC zone (Fig. 1c).

The most common site of involvement for tumors of the left upper division (LUD) ($n = 41$) was the AP or LU zone ($n = 41$; 100 %: Fig. 1e). Left upper division tumors showed a significantly higher incidence of AP or LU zone metastasis than left lower lobe (LLL) tumors (100 vs. 42 %, $p < 0.001$: Fig. 1e, g). In contrast, when AP or LU zone metastasis was present, LLL tumors showed a higher incidence of simultaneous metastasis to the SC or LL zone than LUD tumors, but the difference was not significant (29 vs. 12 %, $p = 0.089$: Fig. 1e, g). The incidence of skip metastasis to only the AP or LU zone was 45 % in left lingular division tumors, 20 % in LUD tumors, and 0 % in LLL tumors, but the difference was not significant (Fig. 1e–g). Left upper division tumors showed a significantly lower incidence of SC zone metastasis than LLL tumors (10 vs. 46 %, $p < 0.001$: Fig. 1h, j). All LUD tumors with SC zone metastasis showed simultaneous metastasis to the AP or LU zone, but no patient showed skip metastasis to the SC zone (Fig. 1h).

Patients were further categorized as those with tumors of the lower lobes ($n = 64$; 40 of right and 24 of left) and those with RUL or LUD tumors ($n = 120$; 79 of RUL and 41 of LUD). The prognosis of patients with lower lobe tumors and RUL or LUD tumors was analyzed. The 5-year overall survival (OS) rates of patients with tumors of the lower lobes with upper mediastinal metastasis ($n = 34$, 22 %) were poorer than, but not significantly different from, those of the patients without upper mediastinal metastasis ($n = 30$, 34 %) ($p = 0.371$; Fig. 2). The 5-year OS rates of patients with RUL or LUD tumors with SC zone metastasis ($n = 16$, 14 %) were poorer than, but not significantly different from, those of the patients without SC zone metastasis ($n = 104$, 40 %) ($p = 0.073$; Fig. 3).

The combined treatment strategies for tumor location-specific SLND in N2 NSCLC patients according to clinical T status are summarized in Table 2. Among 24 patients with upper mediastinal metastasis from RLL tumors, nine showed no evidence of hilar, SC zone, and lower mediastinal metastasis. Of these nine patients, only one had clinical T1. Similarly, among ten patients with upper mediastinal metastasis from LLL tumors, only one showed no evidence of hilar, SC zone, and lower mediastinal metastasis, and clinical T1 status. Upper mediastinal dissection may be unnecessary in lower lobe tumors with negative hilar, SC and lower mediastinal nodes on frozen sections if the preoperative T status is T1 (Table 3). In contrast, among 12 patients with SC zone metastasis from RUL tumors, one showed no evidence of hilar or RU zone metastasis, and that tumor was classified as clinical T2. Among four patients with SC zone metastasis from LUD tumors, none showed evidence of hilar, upper mediastinal metastasis. This finding supports the hypothesis that SC dissection may be unnecessary in RUL and LUD tumors with no metastasis to hilar and upper mediastinal nodes on frozen sections, regardless of the clinical T status. Figure 4 shows diagrams of the main pathways of lymphatic spread of tumors according to tumor location.

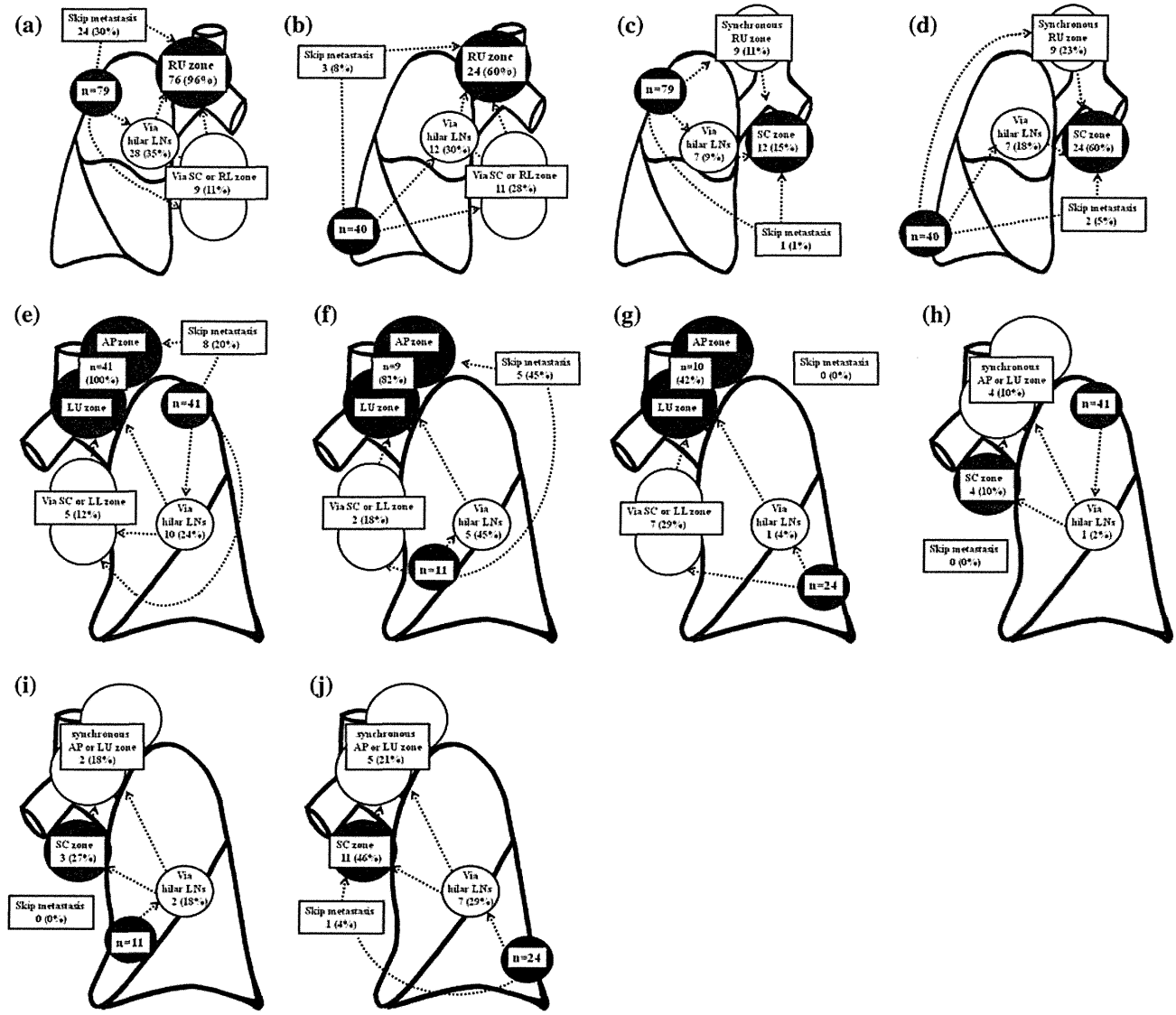

Fig. 1 Lymph node spread patterns according to the primary tumor location: **a** tumors of the right upper lobe (RUL) and right upper mediastinal metastasis. **b** Tumors of the right lower lobe (RLL) and right upper mediastinal metastasis. **c** Tumors of RUL and subcarinal metastasis. **d** Tumors of RLL and subcarinal metastasis. **e** Tumors of the left upper division (LUD) and left upper mediastinal metastasis. **f** Tumors of the left lingular division (LLD) and left upper mediastinal metastasis. **g** Tumors of the left lower lobe (LLL) and left upper mediastinal metastasis. **h** Tumors of LUD and subcarinal metastasis. **i** Tumors of LLD and subcarinal metastasis. **j** Tumors of LLL and subcarinal metastasis

Discussion

We set out to gain insight into the prevalence of lymph node metastasis in each mediastinal region in patients with pN2 NSCLC. The lymphatic pathways by which metastases from primary tumors in various segments and lobes spread toward the hilar and mediastinal lymph nodes have been investigated for over 50 years [12]. Studies of the patterns of location-specific lymphatic pathways of the lung have led to a better understanding of the importance of lymph node staging in the management of lung cancers. Although systematic LND consistently yields precise staging information, it may contradict the concept of the optimal extent of lymph node dissection based on the location of the tumor. Some authors have postulated that the dissection of lymph nodes without cancer cells causes higher morbidity and mortality because it extends the operative procedure [2, 6]. Moreover, the significance of LND regarding long-term outcome is still controversial. We therefore retrospectively reviewed the prevalence of mediastinal lymph node involvement in 207 patients with NSCLC of less than 5 cm with N2 involvement based on the location of the primary tumor, and we set out to determine the possible indications of location-specific SLND.

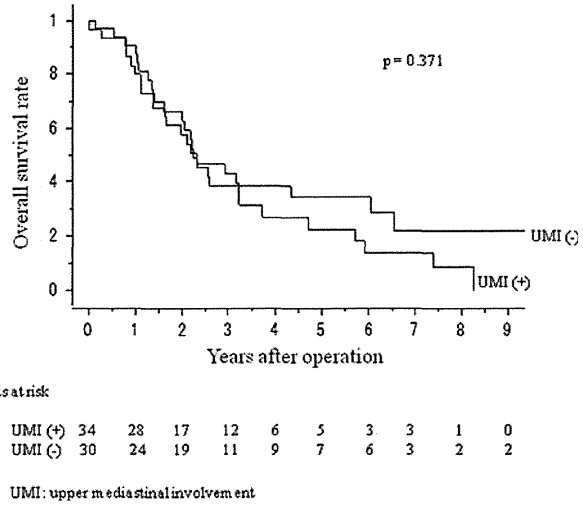

Fig. 2 Overall survival curves of lower lobe non-small cell lung cancer (NSCLC) pN2 patients, with or without upper mediastinal metastasis

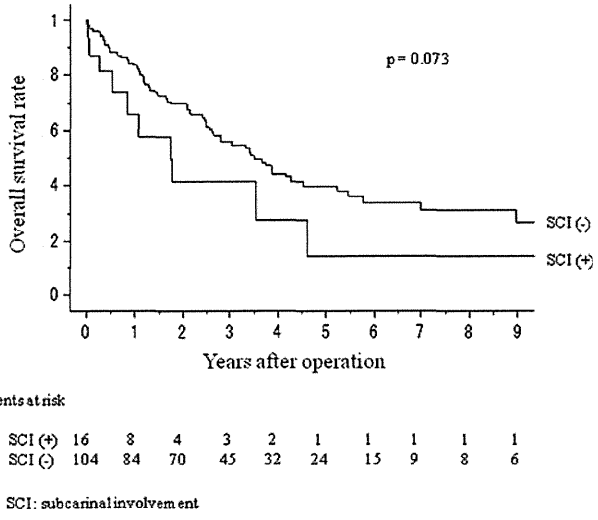

Fig. 3 Overall survival curves of right upper lobe or left upper division NSCLC pN2 patients, with or without subcarinal metastasis

Table 2 Strategy for tumor location-specific selective nodal dissection in N2 non-small cell lung cancer (NSCLC) patients: distribution of upper mediastinal involvement according to clinical T status

Tumor location					
	RUL *n* (%)	RLL	LUD	LLD	LLL
No. of patients with N2	79 (100)	40 (100)	41 (100)	11 (100)	24 (100)
No. of patients with UMI	76 (96)	24 (60)	41 (100)	9 (82)	10 (42)
Patients with UMI					
HI (–), SCI (–), LMI (–)	44 (56)	9 (23)	22 (54)	5 (45)	2 (8)
Clinical T1	14 (18)	1 (4)	5 (12)	2 (18)	1 (4)
Clinical T2–4	30 (38)	8 (21)	17 (41)	3 (27)	1 (4)

RUL right upper lobe, *RLL* right lower lobe, *LUD* left upper division, *LLD* left lingular division, *LLL* left lower lobe, *UMI* upper mediastinal involvement, *HI* hilar lymph node involvement, *SCI* subcarinal involvement, *LMI* lower mediastinal involvement

Table 3 Strategy for tumor location-specific selective nodal dissection in N2 NSCLC patients: distribution of subcarinal involvement according to clinical T status

Tumor location					
	RUL *n* (%)	RLL	LUD	LLD	LLL
No. of patients with N2	79 (100)	40 (100)	41 (100)	11 (100)	24 (100)
No. of patients with SCI	12 (15)	24 (60)	4 (10)	3 (27)	11 (46)
Patients with SCI					
HI (–), UMI (–)	1 (1)	3 (8)	0 (0)	0 (0)	3 (13)
Clinical T1	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
Clinical T2–4	1 (1)	2 (5)	0 (0)	0 (0)	3 (13)

The IASLC staging project proposed the zone classification for future survival analyses [11]. Lee et al. [13] reported that grouping patients together according to zones provides accurate prognostic stratification for patients, and may resolve the ambiguity of the anatomical border, indicating applicability in the clinical setting. Therefore, we used the lymph node zone classification in this study.

Several retrospective studies have shown patterns of mediastinal lymph node metastases in relation to the location of the primary tumor [14–19]. Most of these studies have demonstrated that mediastinal lymph node metastases from RUL tumors occur predominantly in the RU area, but rarely in the SC area, whereas those from left upper lobe tumors occur most frequently in the AP or LU area, but those from tumors of the lower lobes rarely occur in the upper mediastinal area. In the present study, metastases to the SC zone from RUL or LUD tumors were significantly less frequent (15 and 12 %, respectively) than metastases to the SC zone from tumors of the lower lobes. The outcome of patients with RUL or LUD tumors with SC zone metastasis was poorer than, but not significantly different from, that of patients with RUL and LUD tumors without SC zone metastasis ($p = 0.073$). There was only 1 patient with only SC zone skip metastasis. Patients with upper lobe NSCLC involving SC nodes are reportedly rare [16, 18, 19], and they have poorer outcomes than those

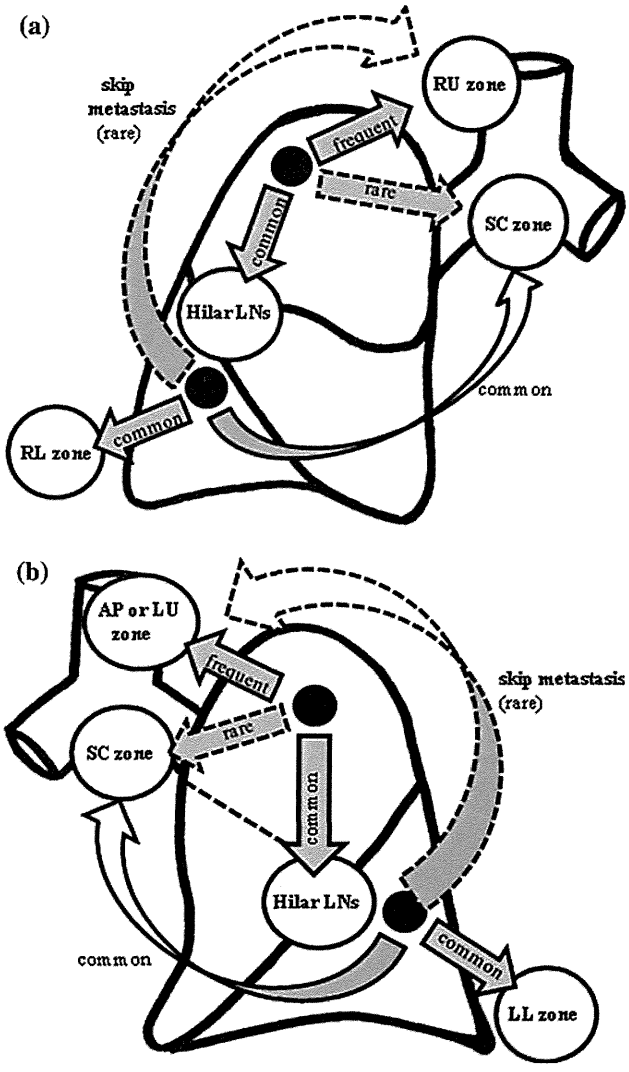

Fig. 4 Diagrams of the main pathways of lymphatic spread according to tumor location. **a** In right-side tumors, almost all RUL tumors metastasized to the RU zone directly or through the hilar lymph node. RUL tumors metastasized less frequently to the SC zone. *Right lower lobe* (RLL) tumors metastasized to various mediastinal lymph node zones, and skip metastasis to the RU zone was rare in RLL tumors. **b** In left-side tumors, all LUD tumors metastasized to the AP zone directly or through the hilar lymph node. *Upper lobe* tumors metastasized less frequently to the SC zone. *Left lower lobe* (LLL) tumors metastasized to various mediastinal lymph node zones, and skip metastasis to the AP zone was rare in LLL tumors

without SC node metastasis [19]. Based on these results, we also evaluated the possible indications of tumor location-specific SLND. Although we did not routinely perform frozen section diagnosis of sampled hilar lymph nodes, we conducted a frozen section examination intraoperatively if metastasis was suspected. There was only 1 patient with SC zone metastasis from RUL tumors who did not show any evidence of hilar and RU zone metastases, whereas no SC zone metastasis from any LUD tumors was observed when neither the hilar nor RU zone showed any evidence of metastasis. Resection of the SC zone in the case of RUL and LUD tumors may be unnecessary if neither upper mediastinal nor hilar lymph nodes show any evidence of metastasis on frozen sections, regardless of the clinical T status.

There were fewer patients with metastases to the upper mediastinal zone from tumors of the lower lobes than with metastases to the upper mediastinal zone from tumors of the upper lobes. The outcome of patients with tumors of the lower lobes with upper mediastinal metastasis was poorer than, but not significantly different from, that of patients with tumors of the lower lobes without upper mediastinal metastasis ($p = 0.371$). There was only one patient each with RU zone metastasis from a clinical T1 RLL tumor and AP zone metastasis from a clinical T1 LLL tumor, but neither showed any evidence of lymph node metastasis to the SC node, lower mediastinal zone, and hilum. Therefore resection of upper mediastinal zones in tumors of the lower lobes may be unnecessary even if the preoperative T status is T1, and if lymph node biopsies in the SC node, lower mediastinal zone, and hilum do not show any evidence of metastasis on frozen sections. However, former studies indicated that the superior and basal segment lung cancers in the lower lobe have different lymph node metastasis patterns [14]. Although there was no significant difference in the metastasis patterns of lower lobe tumors, this finding may be attributable to the small number of patients in the present study (data not shown). The strategy of lymph node dissection should be changed from extensive dissection to SLND, especially in early stage cancer or poor-risk patients, because SLND can reduce postoperative morbidity associated with such complications as bronchopleural fistula, chylothorax, or recurrent nerve palsy [2–5]. However, lung cancer can easily metastasize to the mediastinum, and therefore patient selection should be determined carefully. If patients are suspected of having advanced disease based on intraoperative findings, LND should be performed.

The present study has several limitations. It was a retrospective study, and possible bias may exist. First, we examined suspected hilar or mediastinal lymph nodes intraoperatively in frozen sections, but specific systemic sampling methodologies have been established and used in the past. Second, the number of patients in this study may be too small to draw any definitive conclusion. Third, current less-invasive staging modalities, including PET-CT or EBUS were infrequently used because of the inclusion of a large amount of data from old cases, collected at a time when these procedures were less well established. Thus we might have inadvertently performed some operations on undetected N3 disease.

In conclusion, we demonstrated the potential validity of refraining from resecting lymph nodes in the SC zone in

cases of RUL or LUD tumors, or those in the upper mediastinal zone in the case of tumors of the lower lobes. Considering the fact that NSCLC patients can benefit from SLND, a prospective study is essential to confirm the effect of tumor location-specific SLND on survival and optimal postoperative treatment.

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References

1. Cahan WG (1960) Radical lobectomy. J Thorac Cardiovasc Surg 39:555–572
2. Izbicki JR, Thetter O, Habekost M et al (1994) Radical systematic mediastinal lymphadenectomy in non-small cell lung cancer: a randomized controlled trial. Br J Surg 81:229–235
3. Izbicki JR, Passlick B, Pantel K et al (1998) Effectiveness of radical systematic mediastinal lymphadenectomy in patients with resectable non-small cell lung cancer: results of a prospective randomized trial. Ann Surg 227:138–144
4. Lardinois D, Suter H, Hakki H et al (2005) Morbidity, survival, and site of recurrence after mediastinal lymph-node dissection versus systematic sampling after complete resection for non-small cell lung cancer. Ann Thorac Surg 80:268–274 (discussion 274–265)
5. Okada M, Sakamoto T, Yuki T et al (2006) Selective mediastinal lymphadenectomy for clinico-surgical stage I non-small cell lung cancer. Ann Thorac Surg 81:1028–1032
6. Sugi K, Nawata K, Fujita N et al (1998) Systematic lymph node dissection for clinically diagnosed peripheral non-small-cell lung cancer less than 2 cm in diameter. World J Surg 22:290–294. doi: 10.1007/s002689900384 (discussion 294–295)
7. Darling GE, Allen MS, Decker PA et al (2011) Randomized trial of mediastinal lymph node sampling versus complete lymphadenectomy during pulmonary resection in the patient with N0 or N1 (less than hilar) non-small cell carcinoma: results of the American College of Surgery Oncology Group Z0030 Trial. J Thorac Cardiovasc Surg 141:662–670
8. Henschke CI, McCauley DI, Yankelevitz DF et al (1999) Early Lung Cancer Action Project: overall design and findings from baseline screening. Lancet 354:99–105
9. Sone S, Takashima S, Li F et al (1998) Mass screening for lung cancer with mobile spiral computed tomography scanner. Lancet 351:1242–1245
10. Sobin LH, Gospodarowicz MK, Wittekind C (eds) (2009) TNM classification of malignant tumors. UICC, International Union Against Cancer. Wiley, Weinheim
11. Rusch VW, Crowley J, Giroux DJ et al (2007) The IASLC Lung Cancer Staging Project: proposals for the revision of the N descriptors in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2:603–612
12. Nohl HC (1956) An investigation into the lymphatic and vascular spread of carcinoma of the bronchus. Thorax 11:172–185
13. Lee JG, Lee CY, Bae MK et al (2008) Validity of international association for the study of lung cancer proposals for the revision of N descriptors in lung cancer. J Thorac Oncol 3:1421–1426
14. Watanabe S, Suzuki K, Asamura H (2008) Superior and basal segment lung cancers in the lower lobe have different lymph node metastatic pathways and prognosis. Ann Thorac Surg 85:1026–1031
15. Watanabe S, Asamura H, Suzuki K et al (2005) The new strategy of selective nodal dissection for lung cancer based on segment-specific patterns of nodal spread. Interact Cardiovasc Thorac Surg 4:106–109
16. Okada M, Tsubota N, Yoshimura M et al (1998) Proposal for reasonable mediastinal lymphadenectomy in bronchogenic carcinomas: role of subcarinal nodes in selective dissection. J Thorac Cardiovasc Surg 116:949–953
17. Ishiguro F, Matsuo K, Fukui T et al (2010) Effect of selective lymph node dissection based on patterns of lobe-specific lymph node metastases on patient outcome in patients with resectable non-small cell lung cancer: a large-scale retrospective cohort study applying a propensity score. J Thorac Cardiovasc Surg 139:1001–1006
18. Asamura H, Nakayama H, Kondo H et al (1999) Lobe-specific extent of systematic lymph node dissection for non-small cell lung carcinomas according to a retrospective study of metastasis and prognosis. J Thorac Cardiovasc Surg 117:102–1111
19. Aokage K, Yoshida J, Ishii G et al (2010) Subcarinal lymph node in upper lobe non-small cell lung cancer patients: is selective lymph node dissection valid? Lung Cancer 70:163–167

Number of Circulating Endothelial Progenitor Cells and Intratumoral Microvessel Density in Non-small Cell Lung Cancer Patients

Differences in Angiogenic Status between Adenocarcinoma Histologic Subtypes

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Introduction: Angiogenesis plays a significant role in tumor progression. This study examined the association between the number of circulating endothelial progenitor cells (EPCs), intratumoral microvessel density (MVD) (both of which may be markers for neovascularization), and lung cancer histological types, particularly adenocarcinoma histological subtypes.

Methods: A total of 83 stage I non-small cell lung cancer (NSCLC) patients underwent complete tumor resection between November 2009 and July 2010. The number of EPCs from the pulmonary artery of the resected lungs was measured by assaying $CD34^+$/vascular endothelial growth factor receptor 2 positive cells, and the MVD was assessed immunohistochemically in tumor specimens by staining for CD34.

Results: A statistically significant correlation between the number of EPCs from pulmonary artery and intratumoral MVD was found ($p < 0.001$). No statistically significant differences in the number of EPCs and the MVD were observed between the adenocarcinomas and the squamous cell carcinomas. Among the adenocarcinoma histological subtypes, a higher number of EPCs and MVD were found significantly more frequently in solid adenocarcinomas than in nonsolid adenocarcinomas ($p < 0.001$ and $p = 0.011$, respectively). In addition, solid adenocarcinomas showed higher levels of vascular endothelial growth factor using quantitative real-time polymerase chain reaction in the tumor tissue samples than in the nonsolid adenocarcinomas ($p = 0.005$).

Conclusion: The higher number of circulating EPCs and the MVD of solid adenocarcinoma may indicate the presence of differences in the tumor angiogenic status between early-stage adenocarcinoma histological subtypes. Among adenocarcinoma patients, patients with solid adenocarcinoma may be the best candidates for antiangiogenic therapies.

Key Words: Non-small cell lung cancer, Adenocarcinoma, Subtypes, Angiogenesis, Circulating endothelial progenitor cell.

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Lung cancer is the leading cause of death from cancer in the world,[1] and non-small cell lung cancer (NSCLC) accounts for the majority of lung cancers.[2] Among NSCLC histological types, adenocarcinoma of the lung is the most frequent histological type, and its incidence is increasing in most countries.[3] In Japan, adenocarcinoma is the most common histological type of resected lung cancer, accounting for more than 60% of all cases.[4]

Adenocarcinomas are categorized into four histological subtypes based on morphology: bronchioloalveolar carcinoma (BAC), acinar, papillary, and solid adenocarcinoma.[5] Among these major histological subtypes of lung adenocarcinoma, BAC is often reported to be associated with a favorable prognosis,[6–8] whereas the other subtypes are considered invasive and are associated with unfavorable outcomes.[9,10]

Treatment of lung adenocarcinoma is now moving beyond conventional chemotherapy, with the advent of molecular-targeted therapies such as epidermal growth factor receptor (EGFR) inhibitors.[11] An EGFR mutation was found specifically in the BAC subtype, and differences in the frequency of EGFR mutations exist among the adenocarcinoma histological subtypes.[11]

Angiogenesis is essential for cancer growth and progression.[12] Another key feature of molecular-targeted therapies against lung adenocarcinoma is the inhibition of specific

cytokines essential for tumor vascularization.[13] Although bevacizumab (Avastin; Genentech, South San Francisco, CA), a monoclonal antibody with an antiangiogenic effect that specifically antagonizes and blocks vascular endothelial growth factor (VEGF), had been shown to increase the overall survival of patients with advanced adenocarcinoma when administered in combination with standard chemotherapy,[13] which adenocarcinoma subtypes are most dependent on angiogenesis for their growth remains unclear.

Recent evidence shows that the tumor vasculature can arise through vasculogenesis, a process by which bone marrow–derived $CD34^+$/vascular endothelial growth factor receptor 2 $(VEGFR\text{-}2)^+$ endothelial progenitor cells (EPCs) are recruited and differentiated in situ into mature endothelial cells to form new blood vessels.[14,15] The level of circulating EPCs has been reported to be a potential marker for neovascularization and for the response to antiangiogenic therapies.[16] Other studies have also reported a significant correlation between neovascularization, as assessed by the intratumoral microvessel density (MVD), and patient outcome in a variety of tumors.[17–19]

The addition of antiangiogenic therapy to conventional chemotherapy has recently become a standard therapy for lung adenocarcinoma.[13] However, the exact relationship between specific adenocarcinoma subtypes and the number of EPCs or MVD, both of which may be markers for neovascularization or the response to antiangiogenic therapies, has not been addressed thus far. The evaluation of these relationships could lead to the development of a relatively simple pathological examination of lung adenocarcinoma to determine a patient's sensitivity to antiangiogenic-targeted treatment and may help to identify the biological differences between adenocarcinoma histological subtypes. This study investigated the association between the number of circulating EPCs, MVD, and lung cancer histological types, with particular emphasis on adenocarcinoma subtypes.

PATIENTS AND METHODS

Patients

A total of 122 consecutive Japanese patients with stage I NSCLC underwent complete tumor resection with a lobectomy or a more extensive surgery between November 2009 and July 2010 at the National Cancer Center Hospital East. Among these patients, 39 were excluded because of (1) preoperative chemotherapy and/or radiation therapy ($n = 2$) or (2) unavailability of blood samples from the resected lungs ($n = 37$). The remaining 83 patients were included in this study.

Pathological Evaluation

All surgical specimens were fixed with 10% formalin and embedded in paraffin wax. The tumors were cut at approximately 5-mm intervals, and serial 4-μm sections were stained with hematoxylin-eosin. The disease stages were diagnosed based on the tumor, node, and metastasis (TNM) classification of the International Union Against Cancer, 7th edition.[20] The histological type was determined according to the classification of the World Health Organization.[5] We diagnosed squamous cell carcinoma based on the findings of keratinization, intercellular bridges, and squamous pearl formation. Adenocarcinoma histological subtypes were categorized into BAC (nonmucinous or mucinous), papillary, acinar, and solid adenocarcinomas according to the classification of the World Health Organization.[5] Mucin production in a solid adenocarcinoma component was confirmed using the Alcian blue-periodic acid Schiff method. All the adenocarcinomas were divided according to the predominant subtype into four subgroups: BAC, papillary, acinar, and solid adenocarcinoma with mucin production. Tumor size was measured as the maximal diameter of the tumor on the cut sections. Intratumoral vascular invasion and visceral pleural invasion were evaluated using staining with hematoxylin-eosin and Victoria blue-van Gieson stains.

Blood Sampling from the Pulmonary Artery of Resected Specimens

Human monocytes were isolated from the pulmonary artery (PA) of resected lungs as previously reported.[21,22] In brief, the dissected and ligated PA of surgically resected lungs contains more than 4 ml of blood. In this study, a 21-gauge needle was inserted into the PA of 83 lungs surgically resected from stage I primary NSCLC patients at our hospital. All the specimens were collected after the patients had given their written informed consent, and the study was approved by the Institutional Review Board of the National Cancer Center.

Flow Cytometry

Blood samples from the PA of the resected lungs were processed within 1 hour after collection. Blood mononuclear cells from the PA were prepared by gradient centrifugation using Ficoll-Hypaque. The expression of cell surface antigens was determined using two-color immunofluorescence staining. In brief, 100 μl of blood mononuclear cells (containing 5×10^5 cells) were incubated with 40 μl of FcR-blocking reagent (MBL, Aichi, Japan) for 10 minutes to inhibit nonspecific bindings. Subsequently, the cells were incubated at 4°C for 15 minutes with 10 μl of phycoerythrin-conjugated antihuman CD34 mAb (BioLegend, Bergisch-Gladbach, Germany) and 20 μl of allophycocyanin-conjugated VEGFR-2 mAb (R&D Systems, Wiesbaden-Nordenstadt, Germany). Phycoerythrin (PE)- and allophycocyanin (APC)-conjugated isotype-matched immunoglobulin Ig-G1 (Abcam, Cambridge, United Kingdom) and Ig-G2a (DakoCytomation, Hamburg, Germany) antibodies were used as negative controls. The cells were washed three times to remove unbound antibodies and finally resuspended in 500 μl of fluorescence activated cell sorting (FACS) solution. A FACS analysis was performed using a FACSCalibur flow cytometer (BD Bioscience, Heidelberg, Germany). A minimum of 10,000 events were collected.

Immunohistochemistry

After reviewing the hematoxylin-eosin-stained slides of the surgical specimens, the block containing the most extensive tumor component was selected from each specimen. Sections (4 μm each) were cut from the paraffin blocks and

mounted on silanized slides. The sections were deparaffinized in xylene, dehydrated in a graded ethanol series, washed with distilled water, and placed in 0.1 M citric acid buffer. For antigen retrieval, the slides were heated at 95°C for 20 minutes in a microwave oven and then allowed to cool for 1 hour at room temperature. Next, the slides were washed three times in phosphate-buffered saline (PBS) and immersed in a 0.3% hydrogen peroxide solution in methanol for 15 minutes to inhibit endogenous peroxidase activity. After washing the slides three times in PBS, nonspecific binding was blocked by preincubation with 2% normal swine serum in PBS (blocking buffer) for 30 minutes at room temperature. Individual slides were then incubated overnight at 4°C with mouse anti-CD34 antibody (R&D Systems) at a final dilution of 1:50 in blocking buffer. The slides were again washed three times with PBS, incubated with EnVision (DAKO, Tokyo, Japan) for 1 hour at room temperature, and after extensive washing with PBS, the color reaction was developed for 2 minutes in 2% 3,3′-diaminobenzidine in 50 mM Tris-buffer (pH 7.6) containing 0.3% hydrogen peroxide. Finally, the sections were counterstained with Meyer's hematoxylin, dehydrated, and mounted. The three most vascular areas (hot spots) in the invasive foci within a section were selected for the quantification of angiogenesis, and vessels labeled with the anti-CD34 mAb were counted under light microscopy at a magnification of ×400 (×400; ×40 objective, and ×10 ocular; 0.196 mm^2/field) based on previous reports.[18–23] Each single or connected endothelial cell that stained in these areas was counted as a microvessel. The average counts were recorded as the CD34-MVD for each case.

Tissue Samples, RNA Extraction, Reverse Transcription, and Real-Time Polymerase Chain Reaction

Total RNA was extracted from 27 adenocarcinoma patients who had undergone surgical resection at our hospital. Samples of cancer tissue were collected and immediately homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) with Multi-Beads Shocker (Yasui Kikai, Osaka, Japan) and stored at −80°C until use. Total RNA was isolated from the tissues using a commercial RNA isolation reagent according to the manufacturer's instructions. The RNA was reverse transcribed to synthesize cDNA using a PrimerScript RT reagent kit according to the manufacturer's instructions (Takara Biochemicals, Shiga, Japan).

To quantitatively compare the mRNA level of VEGF-A, RT-PCR was performed in a Smart Cycler System (TaKaTa) using SYBR Premix Ex Taq (TaKaRa). The sense and antisense primers used for the quantitative amplification of VEGF mRNAs were 5′-GAGCCTTGCCTTGCTGCTC-TAC-3′ and 5′-CACCAGGGTCTCGATTGGATG-3′, and the primers used for the amplification of glyceraldehyde-3-phosphate dehydrogenase as an internal control were 5′-GCACCGTCAAGGCTGAGAAC-3′ and 5′-ATGGTGGT-GAAGACGCCAGT-3′.

The amount of template cDNA was expressed by a threshold cycle (G) that was determined from the amplification curve (exponential curve) and a threshold level of PCR product detection. One G was equal to a twofold difference in the initial template. The quantification data were analyzed using Smart Cycler System software, version 2.0d (Cepheid). The level of VEGF expression was reported as the ratio of its expression to the level of *GAPDH* gene expression in the same sample.

Clinicopathological Data

The medical records of all the patients were reviewed to obtain the clinicopathological data, which included age (dichotomized according to a median age of 69 years), sex, smoking history (nonsmokers or ever-smokers), diameter of the tumor on the resected specimens (dichotomized according to a diameter of 3.0 cm), histological type (adenocarcinoma, squamous cell carcinoma, or others), lymphatic permeation (present or absent), intratumoral vascular invasion (present or absent), and visceral pleural invasion (as defined in the TNM classification, 7th edition[17]; present or absent).

Data and Statistical Analysis

All data are presented as the mean ± SE. Differences in categorical outcomes were evaluated using the χ^2 test. Continuous variables were compared using t tests. All the reported p values were two-sided, and the significance level

TABLE 1. Patient Characteristics

Characteristics	No. of Patients (%)
Total	83 (100)
Age (yr)	
<69	41 (49)
≥69	42 (51)
Sex	
Women	30 (36)
Men	53 (64)
Smoking history	
Never-smoker	32 (39)
Ever-smoker	51 (61)
Tumor size (cm)	
≤3	62 (75)
>3	21 (25)
Histologic type	
Adenocarcinoma	63 (76)
Predominant subtype	
BAC	23
Papillary	12
Acinar	18
Solid	10
Squamous cell carcinoma	15 (18)
Others	5 (6)
Lymphatic permeation	
Absent	77 (93)
Present	6 (7)
Intratumoral vascular invasion	
Absent	59 (71)
Present	24 (29)
Visceral pleural invasion	
Absent	69 (83)
Present	14 (17)

BAC, bronchioloalveolar carcinoma.

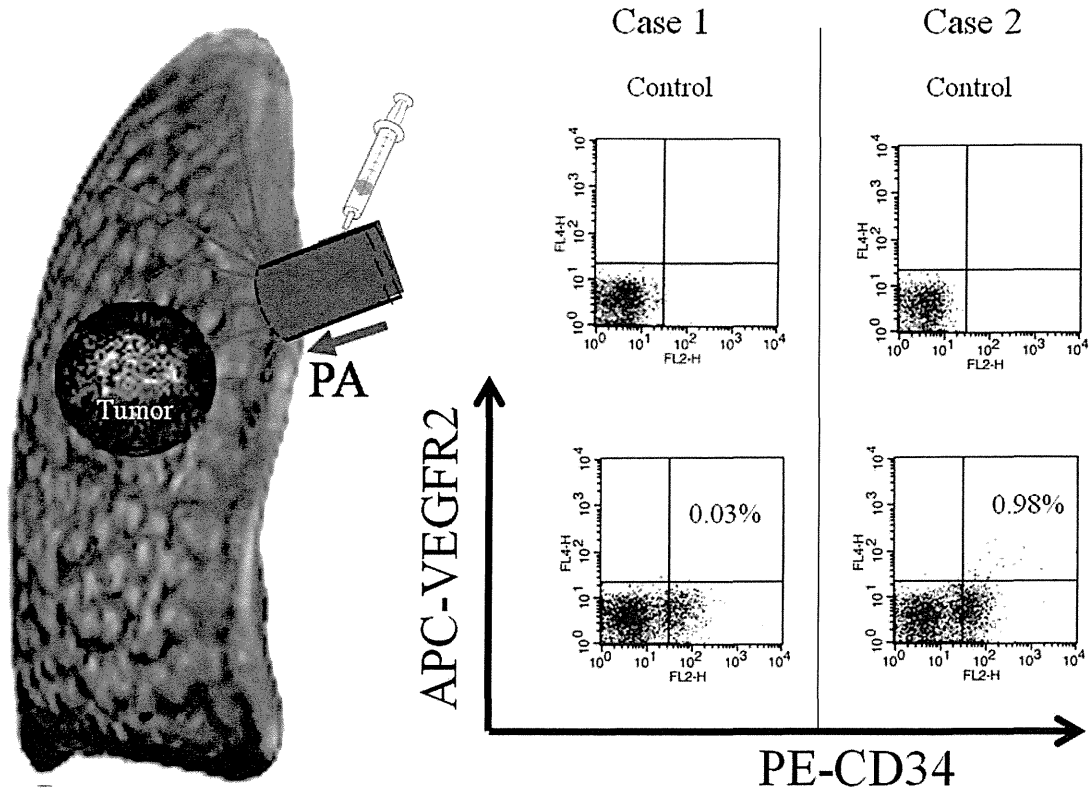

FIGURE 1. Quantification of circulating endothelial progenitor cells (EPCs) in blood mononuclear cells from the pulmonary fraction. Representative data from two flow cytometry analyses are shown. PA, pulmonary artery; VEGFR2, vascular endothelial growth factor receptor 2; APC, allophycocyanin; PE, phycoerythrin.

was set at less than 0.05. Analyses were performed using SPSS version 11.0 (Dr. SPSS II for Windows, standard version 11.0; SPSS Inc., Chicago, IL).

RESULTS

Patient Characteristics

This study analyzed 83 patients with completely resected stage I NSCLC, including 53 men and 30 women with a median (range) age of 69 years (42–82 years). Thirty-two patients were never-smokers, and 51 were ever-smokers. The patient characteristics are shown in Table 1.

Sixty-three patients had adenocarcinoma, 15 had squamous cell carcinoma, and 5 had other histological subtypes. The 63 patients with adenocarcinoma were classified according to the predominant subtypes as follows: 23 patients had BAC, and 12 patients had papillary, 18 patients had acinar, and 10 patients had solid adenocarcinomas. Of the 83 patients included in this study, 21, 6, 24, and 14 patients had tumors with a large tumor size (≥3 cm), lymphatic permeation, intratumoral vascular invasion, and pleural invasion, respectively (Table 1).

EPCs from PA in Stage I NSCLC and Correlation between Clinicopathological Characteristics and the Number of EPCs

Figure 1 shows the EPC quantification in blood mononuclear cells from the PA fraction and representative data for two flow cytometry analyses. Table 2 shows the number of EPCs from the PA fraction according to the clinicopathological features in all the patients. No statistically significant correlations were observed between the number of EPCs and the patient's age, sex, smoking history, tumor size, histological types, lymphatic permeation, vascular invasion, or visceral pleural invasion (Table 2).

MVD in Stage I NSCLC and Correlation between Clinicopathological Characteristics and MVD

The median microvessel count for all patients was 16.2 (per 400× field), with an interquartile range of 0 to 78.7. Representative tissue specimens from stage I NSCLC patients are shown in Figure 2 for adenocarcinomas with a low MVD (Figure *2A*) and a high MVD (Figure *2B*) and squamous cell carcinomas with a low MVD (Figure *2C*) and a high MVD (Figure *2D*). Patients were classified into two groups with a high MVD (>16.2) or a low MVD (≤16.2) based on the median MVD of the entire group.

Table 3 lists the MVD in correlation with the clinicopathological features in all the patients. No statistically significant correlations were observed between the MVD and the patient's age, sex, smoking history, tumor size, histological type, lymphatic permeation, vascular invasion, or visceral pleural invasion. In contrast, a statistically significant corre-

TABLE 2. Correlation between Clinicopathological Characteristics and the Number of EPCs in the Entire Cohort

Characteristics	No. of Patients (%)	No. of EPC from PA (/ml) ± SE	*p*
Total	83 (100)	2619 ± 372	
Age (yr)			
<69	41 (49)	2829 ± 569	0.580
≥69	42 (51)	2414 ± 486	
Sex			
Women	30 (36)	1730 ± 315	0.072
Men	53 (64)	3122 ± 546	
Smoking history			
Never-smoker	32 (39)	1768 ± 342	0.069
Ever-smoker	51 (61)	3153 ± 556	
Tumor size (cm)			
≤3	62 (75)	2456 ± 409	0.454
>3	21 (25)	3100 ± 846	
Histologic type			
Adenocarcinoma	63 (76)	2420 ± 397	
Squamous cell carcinoma	15 (18)	3527 ± 1204	0.272[a]
Others	5 (6)	2403 ± 429	0.991[a]
Lymphatic permeation			
Absent	77 (93)	2459 ± 358	0.123
Present	6 (7)	4678 ± 2345	
Intratumoral vascular invasion			
Absent	59 (71)	2193 ± 356	0.072
Present	24 (29)	3667 ± 923	
Visceral pleural invasion			
Absent	69 (83)	2480 ± 389	0.409
Present	14 (17)	3306 ± 1105	

[a] Compared with adenocarcinoma.
EPC, circulating endothelial progenitor cell; PA, pulmonary artery.

lation was observed only between the MVD and the number of EPCs ($p < 0.001$). In the high MVD group, the number of EPCs was significantly higher than that in the low MVD group (mean = 4380 ± 620 and 901 ± 185, respectively; Figure 3).

Correlation between the Number of EPCs and Adenocarcinoma Histological Subtypes

Figure 4*A* shows the number of EPCs in stage I adenocarcinoma patients stratified according to their predominant histological subtypes. The number of EPCs in patients with predominantly solid adenocarcinomas (mean = 5776 ± 1720/ml) was significantly higher than that among patients with predominantly BAC (mean = 1643 ± 420/ml, $p = 0.003$), papillary adenocarcinoma (mean = 2140 ± 770/ml, $p = 0.048$), or acinar adenocarcinoma (mean = 1734 ± 394/ml, $p = 0.007$).

No statistically significant differences in the number of EPCs were observed among patients with predominantly BAC, papillary adenocarcinoma, and acinar adenocarcinoma. Therefore, patients with predominantly BAC, papillary adenocarcinoma, or acinar adenocarcinoma were grouped as nonsolid adenocarcinomas patients and were compared with patients with predominantly solid adenocarcinomas in the following analyses (Figure *4B*).

Differences in MVD and VEGF Levels in Tumor Tissue Samples between Solid and Nonsolid Adenocarcinoma Patients as Determined Using Quantitative Real-Time PCR

Table 4 shows the differences in the MVD between solid and nonsolid adenocarcinoma patients. The high-MVD group included a significantly higher number of solid adenocarcinoma patients than those with nonsolid adenocarcinoma patients ($p = 0.011$).

Because VEGF-A has been reported to play a crucial role in the recruitment of EPCs[24,25] and angiogenesis can be assessed according to the MVD,[26,27] the level of mVEGF-A

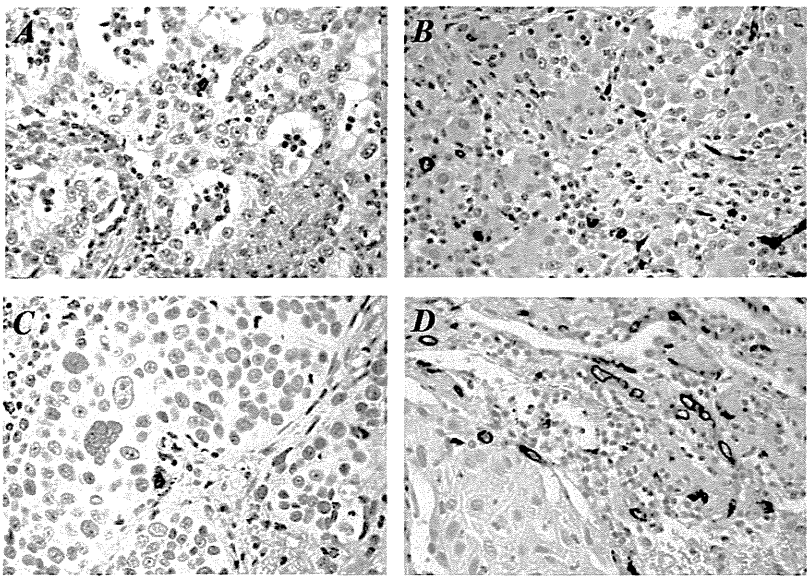

FIGURE 2. Immunohistochemical staining of non-small cell lung cancer (NSCLC) tissue with anti-CD34 antibodies for the quantification of microvessel density (MVD). Sections from specimens of adenocarcinoma with a low MVD (*A*) and a high MVD (*B*) and squamous cell carcinoma with a low MVD (*C*) and a high MVD (*D*) are shown. Original magnification, ×400. EPC, endothelial progenitor cell; PA, pulmonary artery.

TABLE 3. Correlation between Clinicopathological Characteristics and the Number of EPCs and MVD in the Entire Cohort

Characteristics	No. of Patients (%)	MVD		p
		Low[a]	High[b]	
Total	83 (100)	42	41	
Age (yr)				
<69	41 (49)	18 (44)	23 (56)	0.228
≥69	42 (51)	24 (57)	18 (43)	
Sex				
Women	30 (36)	17 (57)	13 (43)	0.406
Men	53 (64)	25 (47)	28 (53)	
Smoking history				
Never-smoker	32 (39)	19 (59)	13 (41)	0.205
Ever-smoker	51 (61)	23 (45)	28 (55)	
Tumor size (cm)				
≤3	62 (75)	32 (52)	30 (48)	0.752
>3	21 (25)	10 (48)	11 (52)	
Histologic type				
Adenocarcinoma	63 (76)	36 (57)	27 (43)	0.093
Squamous cell carcinoma	15 (18)	5 (33)	10 (67)	
Others	5 (6)	1 (20)	4 (80)	
Lymphatic permeation				
Absent	77 (93)	40 (52)	37 (48)	0.38
Present	6 (7)	2 (33)	4 (67)	
Intratumoral vascular invasion				
Absent	59 (71)	33 (56)	26 (44)	0.128
Present	24 (29)	9 (38)	15 (62)	
Visceral pleural invasion				
Absent	69 (83)	37 (54)	32 (46)	0.222
Present	14 (17)	5 (36)	9 (64)	
No. of EPC from PA/ml ± SE	83 (100)	901 ± 185	4380 ± 620	<0.001[c]

Numbers in parentheses are in percentages.
[a] ≤median.
[b] >median.
[c] Significance.
MVD, microvessel density; EPC, circulating endothelial progenitor cell; PA, pulmonary artery.

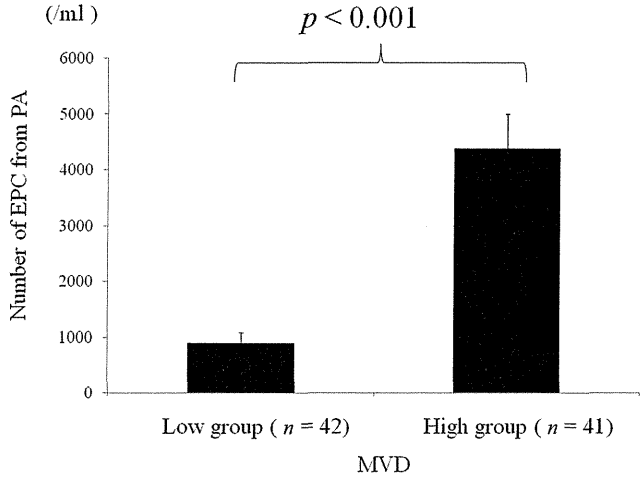

FIGURE 3. Correlation between the microvessel density (MVD) and the number of endothelial progenitor cells (EPCs) in the entire cohort. PA, pulmonary artery.

was measured using quantitative real-time PCR in the tumor tissue samples and was compared between solid and nonsolid adenocarcinoma patients. The level of mVEGF-A was significantly higher among solid adenocarcinomas than among nonsolid adenocarcinomas (Figure 5, $p = 0.005$).

DISCUSSION

In the most recently reported series, adenocarcinoma was found to be the most common type of early-stage lung cancer.[3,4] The recent increase in the detection of early-stage adenocarcinoma in Japan can be attributed to a nationwide mass screening system.[28] The major histological subtypes of adenocarcinoma are characterized as BAC, acinar, papillary, and solid components. Several reports have described differences in survival between adenocarcinoma subtypes. BAC has specific radiological features and is reported to be significantly correlated with a favorable prognosis.[6–8] In contrast, patients with solid adenocarcinoma have significantly poorer outcomes than those with other histological subtypes, and solid adenocarcinoma is

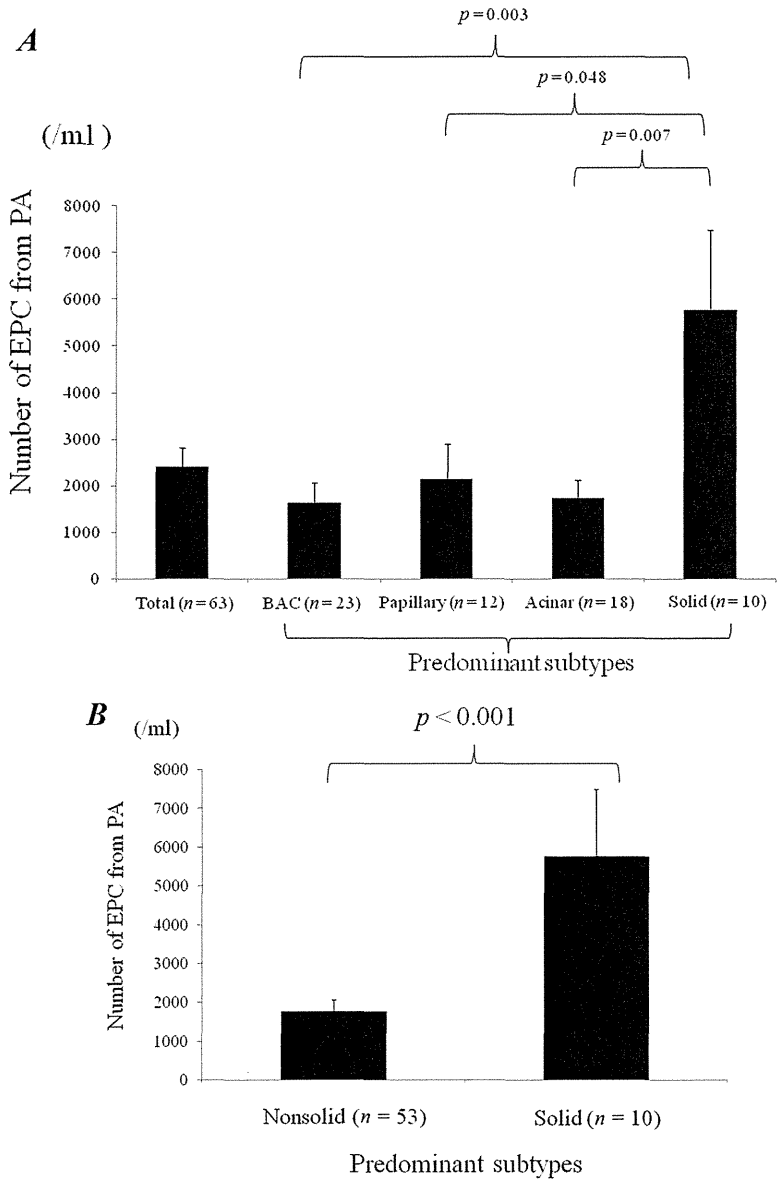

FIGURE 4. *A*, Correlation between the number of endothelial progenitor cells (EPCs) and adenocarcinoma histological subtypes. *B*, Differences in number of endothelial progenitor cells (EPCs) between solid and nonsolid adenocarcinoma patients. PA, pulmonary artery; BAC, bronchioloalveolar carcinoma.

TABLE 4. Differences in MVD between Solid and Nonsolid Adenocarcinoma Patients

Predominant Adenocarcinoma Subtypes	No. of Patients (%)	MVD		*p*
		Low[a]	High[b]	
Total	63	36 (57)	27 (43)	
Nonsolid subtypes	53 (84)	34 (64)	19 (36)	
Solid subtype	10 (16)	2 (20)	8 (80)	0.011[c]

Numbers in parentheses are in percentages.
[a] ≤median in the entire cohort.
[b] >median in the entire cohort.
[c] Significance.
MVD, microvessel density.

the most poorly differentiated among lung adenocarcinoma subtypes.[9,10] In addition, Ding et al.[11] reported that an EGFR mutation showed a significant positive correlation with BAC and papillary subtypes but not with the solid subtype. Thus, with the introduction of computed tomography screening and the discovery of targeted small molecule therapies against EGFR, there has been an enormous interest in the pathology, radiology, molecular biology, and clinical features of lung adenocarcinoma subtypes.

Several reports have revealed that angiogenesis plays a significant role in the pathogenesis of tumors and in the mechanisms of disease progression.[12,18] Recent reports have indicated that the transplantation of ex vivo cultivated EPCs reportedly contributes to angiogenic tumor vasculature.[29–31] Despite certain discrepancies in the existing reports,[32–34] the role of EPCs in vessel formation in tumors has now become widely accepted. Therefore, recent evidence demonstrating