Case presentation

Clinical summary

Case1

A 49-year-old Japanese man was referred to the hospital with lung adenocarcinoma, which was diagnosed by the transthoracic needle biopsy. A computed tomography (CT) scan detected a 32 mm-sized localized solid tumor in the right upper lobe and swelling of the mediastinal lymph node (Figure 1a). He was an ex-smoker and admission laboratory tests showed increased carcinoembryonic antigen (9.6 ng/mL). A right upper lobectomy with lymph node dissection was performed and the tumor was diagnosed as lung adenocarcinoma in pathological T2aN2M0 and stage IIIA determined on the basis of the TNM classification of Union of International Cancer Control [20]. After that, he underwent postoperative adjuvant chemotherapy, and he was alive without recurrence ten months after operation.

Case2

A 57-year-old Japanese man who was a never smoker was referred to the hospital with abnormal shadow on his chest CT scan. A CT scan detected a 29 mm-sized localized solid nodule with pleural indentation in the right upper lobe, and the histological diagnosis of the tumor by transbronchoscopic biopsy was lung adenocarcinoma (Figure 1b). Laboratory tests showed slightly elevated squamous cell carcinoma antigen (1.6 ng/mL). A right upper lobectomy with lymph node dissection was performed and diagnosed lung adenocarcinoma in pathological T2aN0M0 and stage IB. After that, he underwent postoperative adjuvant chemotherapy, and he was alive without recurrence nine months after operation.

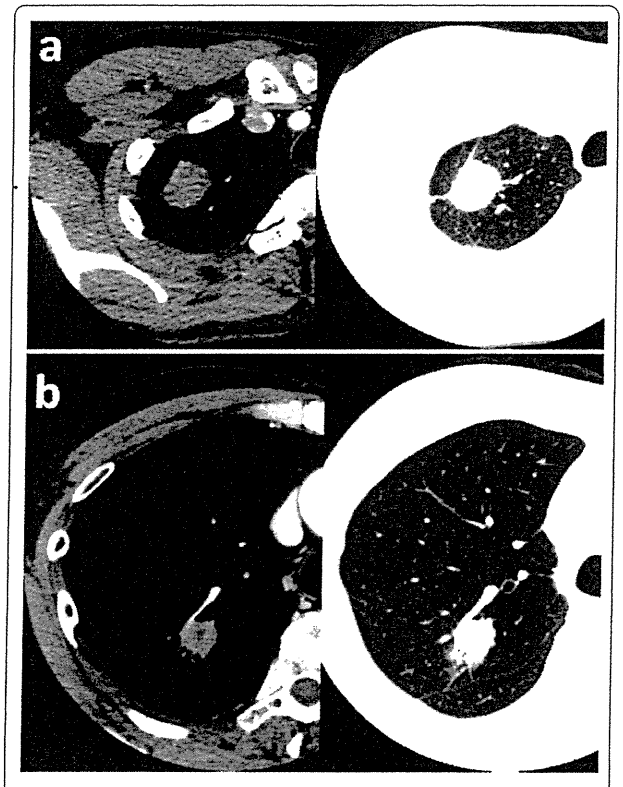

Figure 1 Enhanced chest CT of the lung. Chest CT of case 1 (a) and case 2 (b) showed a tumor of the right upper lobe of the lung. (a) Chest CT revealed a tumor with pleural indentation, without ground glass opacity (GGO). The tumor was 32 mm in diameter and mildly enhanced. (b) Chest CT revealed a nodule with GGO, pleural indentation, air bronchogram and venous involvement. The nodule was 29 mm in diameter and mildly enhanced.

Pathological findings

The excised specimens were fixed in a solution of 10% buffered formaldehyde and the sections were embedded in paraffin. Four micrometer-thick sections including the largest cut surface of the tumor were prepared and stained with hematoxylin and eosin (HE), alcian blue and elastica-van-Gieson (AB-EVG) stain. Immunohistochemical staining was performed with the primary antibodies listed in Table 1. Lepidic, acinar, papillary and solid subtypes of lung adenocarcinoma were determined according to the 2004 WHO classification. AMP of lung adenocarcinoma was determined according to the IASLC/ATS/ERS international multidisciplinary classification of lung adenocarcinoma [1].

Macroscopic examination of the excised specimens showed the sharply-demarcated tumor measuring 36 × 25 × 30 mm in size, white in color on cut surface in the case 1 (Figure 2a), and the tumor measuring 33 × 20 × 40 mm in size, grayish white in color on cut surface in the case 2 (Figure 2b).

Microscopically, the tumors of both the cases were composed of stromal micropapillary predominant adenocarcinoma, which were proliferation of nonmucinous atypical cuboidal epithelial cells. In the case 1, the tumor was composed of 55% SMP, 5% lepidic, 30% acinar, 5% papillary pattern and 5% AMP (Figure 3a). In the case 2, the tumor was composed of 55% SMP, 20% lepidic and 25% acinar pattern (Figure 3b) and lacked AMP. In both the cases, lymphatic and vessel invasion were often observed. Lymphatic invasion was confirmed on immunohistochemistry using D2-40 antibody. The case 1 showed pleural invasion and resected regional lymph nodes had metastatic foci composed of tumor cells with a micropapillary pattern (Figure 4).

Immunohistochemically, the outer surface of the SMP cell clusters in both the cases showed membranous expression of MUC1 and epithelial membrane antigen (EMA), indicating an 'inside-out' pattern. Both the cases showed E-cadherin expression on intercellular cell membranes of micropapillary tufts of SMP tumor cells. Tumor cells constituting SMP showed positive staining for Ki-67 (MIB-1, Dako, Glostrup, Denmark); positive

Table 1 Antibodies used and immunohistchemical result

Antibody	Clone	Dilution	Source	Stroma around the micropapillary clusters		Tumor cells			
						in SMP		outside SMP	
				Case1	Case2	Case1	Case2	Case1	Case2
MUC1	Ma695	1:100	Novocastra	0	0	2+	2+	1+	2+
EMA	E29	Pre-diluted	Cell Marque	0	0	2+	2+	2+	2+
E-cadherin	NCH38D	1:100	DakoCytomation	0	0	2+	2+	2+	2+
CK	AE1/AE3	Pre-diluted	Nichirei	0	0	2+	2+	2+	2+
TTF-1	8G7G3/1	1:100	DakoCytomation	0	0	1+	2+	2+	2+
SP-A	PE10	1:100	Dako	0	0	1+	0	2+	2+
D2-40	D2-40	Pre-diluted	Nichirei	0	0	0	0	0	0
CD34	Nu-4A1	Pre-diluted	Nichirei	0	0	0	0	0	0
FactorVIII	polyclonal	1:200	DAKO	0	0	0	0	0	0

EMA, epithelial membrane antigen; CK, cytokeratin; TTF-1, thyroid transcription factor-1; SP-A, surfactant protein A.

Novocastra, Newcastle Upon Tyne, UK; Cell Marque, Hot springs, AR, USA; DakoCytomation, Carpinteria, CA, USA; Dako, Kyoto, Japan; Nichirei, Tokyo, Japan; DAKO, Glostrup, Denmark.

0, no positive cells; 1+, positive tumor cells less than 10%; 2+, 10% or more.

rate were 24% in case 1, 62% in case 2. In SMP component, positive and negative tumor cells for thyroid transcription factor-1 (TTF-1) and surfactant protein A (SP-A) staining were observed in the case 1, on the other hand, the case 2 showed TTF-1 expression and SP-A repression in almost all tumor cells. Tumor cells outside SMP showed MUC1 and EMA expression on free surface of cell membrane, but MUC1 positive cells were fewer than those in SMP. Tumor cells outside SMP in case 1 showed strong TTF-1 expression. D2-40, CD34 and factor VIII were not found in cells constituting micropapillary tufts. Moreover TTF-1, cytokeratin (CK), D2-40, CD34 and factor VIII were negative in luminal inner surface surrounding micropapillary tufts (Table 1 Figure 5a-d). These results means micropapillary tufts of SMP lack fibrovascular core and were not located within alveolar space, vascular or lymphatic vessels.

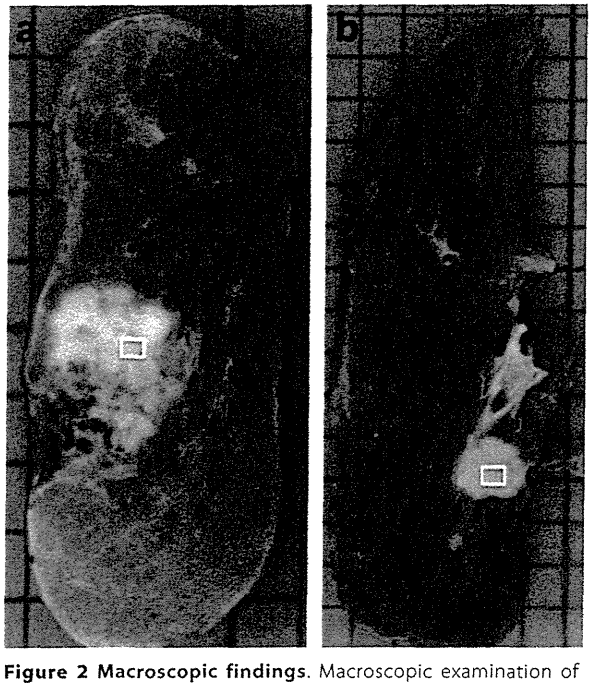

Figure 2 Macroscopic findings. Macroscopic examination of excised specimens showed the tumor measuring 36 × 25 × 30 mm in size, white in color on cut surface in the case 1 (a), and the tumor measuring 33 × 20 × 40 mm in size, grayish-white in color on cut surface containing areas of hemorrhage in the case 2 (b). White squares in Figure 2a and 2b correspond to photomicrographs of Figure 3a and 3b, respectively.

Mutational analysis

A study of epidermal growth factor receptor (*EGFR*) gene status of exon 19 and 21 was performed by the methods described elsewhere [21]. Written informed consent for genetic analysis of the tumor cells was obtained from the patients. As a result, both the cases showed an L858R point mutation at exon 21.

Discussion

In the two cases presented, we recognized that tumor cells of SMP had reduced expression of SP-A, but tumor cells outside SMP had its strong expression. Additionally, we identified the tumor cluster cells were surrounded by the connective tissue which was negative for TTF-1 or CK. And these components were also negative for D2-40 and CD34. The results suggested micropapillary tufts of SMP were not located within alveolar space, vascular or lymphatic vessels but in stroma and reduced the phenotypic expression like SP-A [22].

AMP of MSLA has been reported to express of MUC1 [6]. We identified expression of MUC1 and EMA in the outer surface of papillary clusters like those in AMP of the case 1. This staining pattern is called as an 'inside-out' pattern in the invasive micropapillary carcinoma of

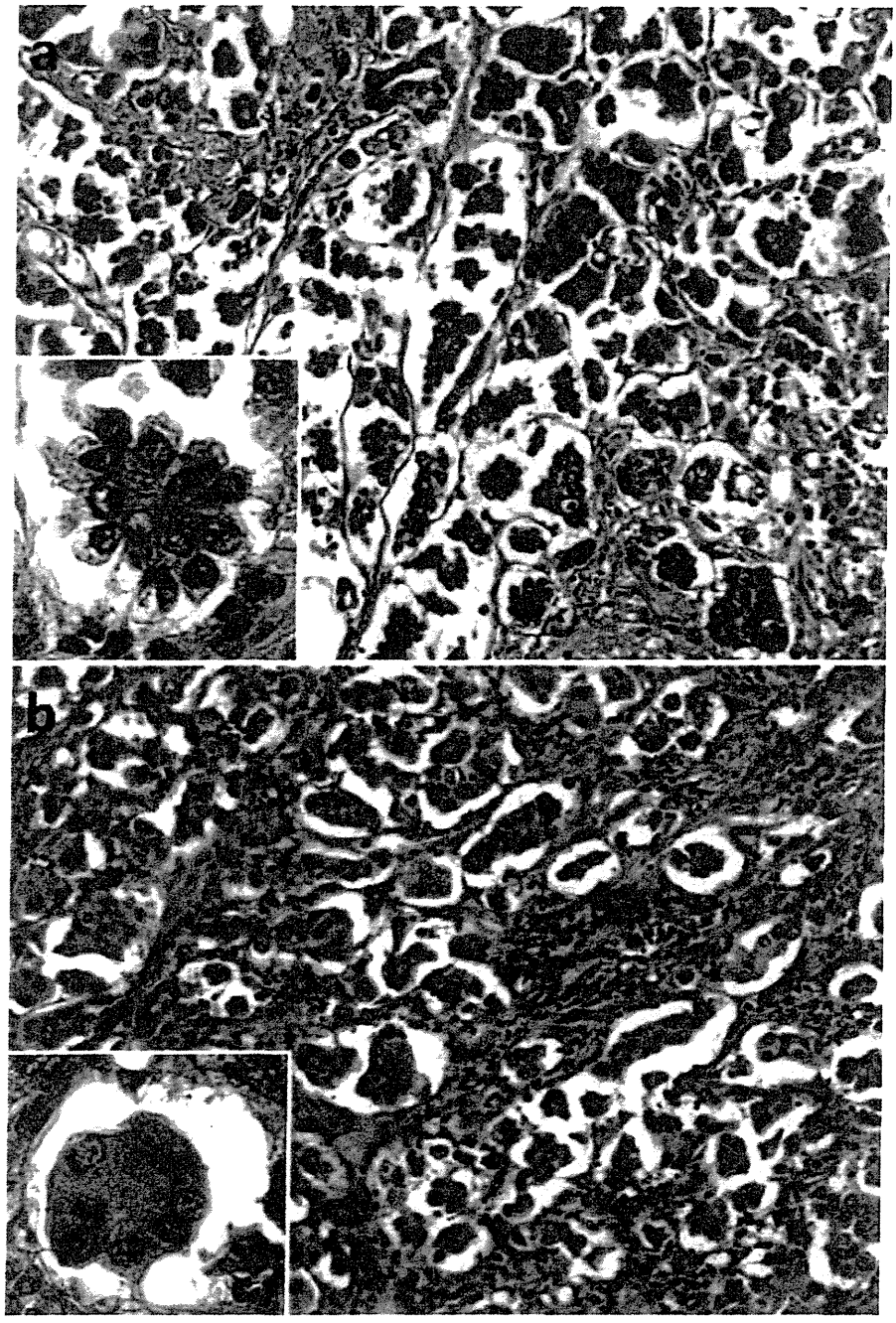

Figure 3 Photomicrographs. Stromal micropapillary pattern characterized by papillary structures with tufts lacking a central fibrovascular core and surrounding clear spaces in fibrotic stroma was seen in both case 1 (a) and case 2 (b). (HE stains ×100; inset, ×400)

breast [7,8] and several organs [9-19]. Therefore, the polarity called as an 'inside-out' pattern of the tumor cells is thought to be a characteristic feature in SMP and AMP of lung adenocarcinoma.

Previous studies have reported that a micropapillary pattern is associated with a poor prognosis in stage I lung adenocarcinoma because of its aggressive behavior, as shown by frequent lymph node metastasis, lymphatic permeation, vascular invasion and pleural invasion [4-6]. In the current two cases, lymphatic permeation and vascular invasion were often observed. In addition, the case 1 showed pleural invasion and the resected regional

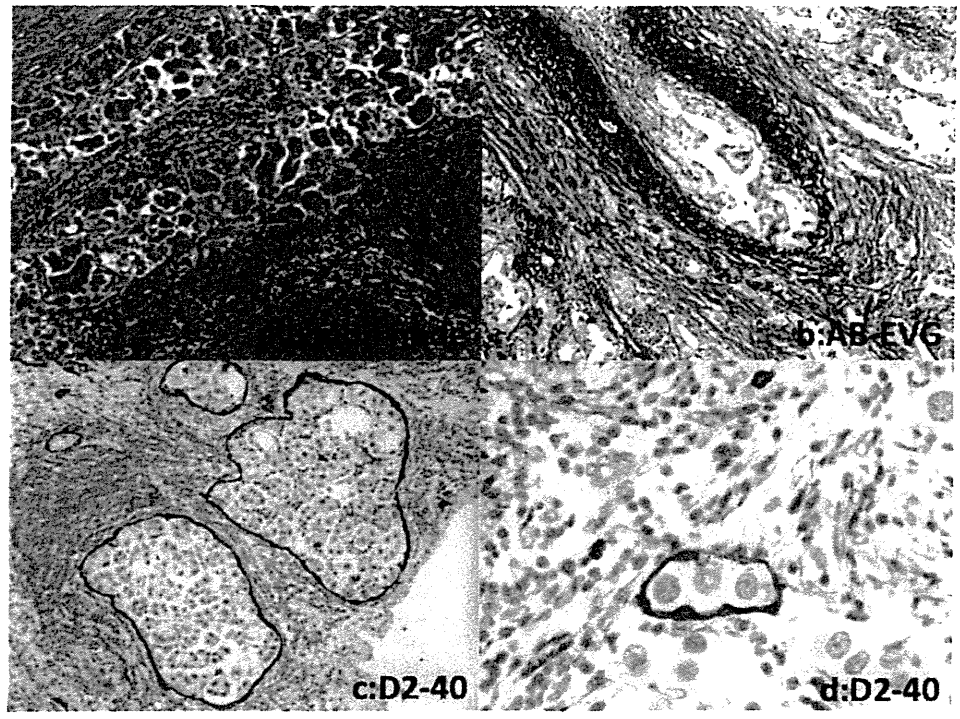

Figure 4 Photomicrographs. (a) A resected regional lymph node had metastatic foci composed of tumor cells with a micropapillary pattern in the case 1. (HE stain, ×200) (b) Vessel invasion by tumor cells in the case 1. (AB-EVG stain, ×100) (c, d) Tumor cells in lymphatic ducts, which were covered by D2-40 positive endothelial cells. (c, ×200, case 1; d, ×400, case 2).

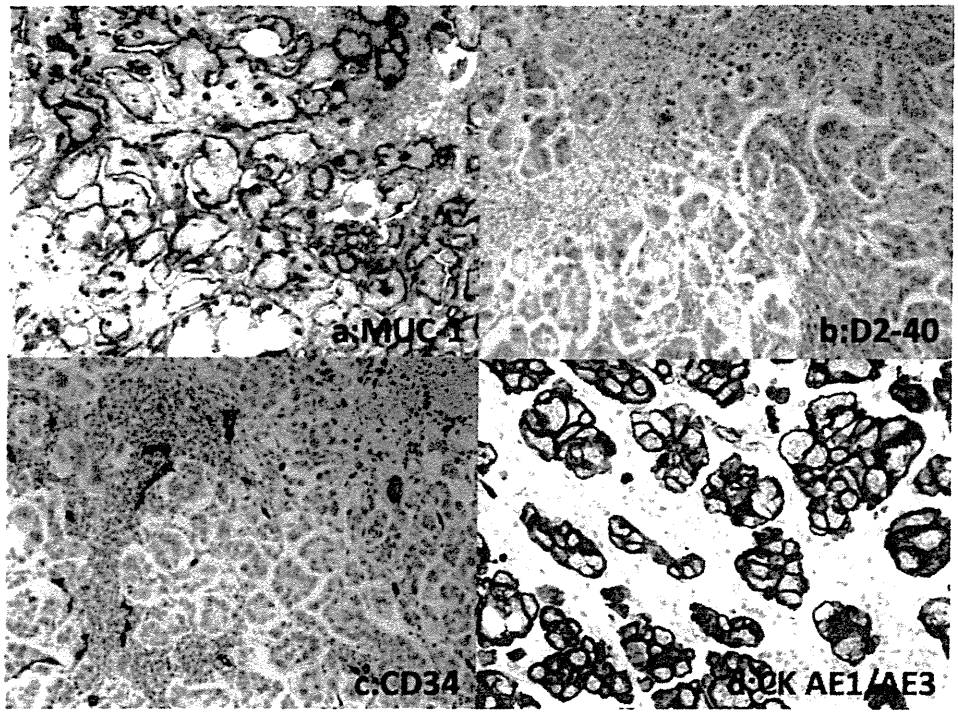

Figure 5 Immunohistochemical photomicrographs of case 1. (a) Tumor clusters of stromal micropapillary pattern had MUC1 expression on the outer surface strongly, indicating 'inside-out' pattern. (×200) (b) D2-40 and (c) CD34 were not found in cells consisting micropapillary tufts and (d) CK was not found in connective tissue surrounding the tumor cells. (b, c, ×200; d, ×400).

lymph node had metastatic foci composed of tumor cells with a micropapillary pattern. Though it is too early to refer to prognosis of SMPPLA because of short duration of observation, we may say SMPPLA has strong association with vascular invasion.

We also performed a mutational analysis of *EGFR* gene mutations and both the cases showed *EGFR* mutations of exon 21. These results suggest that SMPPLA might be associated with poor prognosis and effective for EGFR tyrosine kinase inhibitors.

In conclusion, we recognized the presence of SMPPLA. Since SMPPLA is very rare as far as we investigated, further studies are required to determine the clinical significance of SMPPLA in detail.

Consent

Written informed consent was obtained from the patients for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Abbreviations

AMP: aerogenous micropapillary pattern; SMP: stromal micropapillary pattern; SMPPLA: SMP predominant lung adenocarcinoma; MSLA: the micropapillary subtype of lung adenocarcinoma

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Authors' contributions

MO and TY designed the study, performed clinical and pathological investigation, and wrote the drafts. YS participated in pathological and genetical investigation. SO performed the histological and immunohistochemical evaluation. CH, KW, KN, TW and RH assisted the clinical investigation. HN participated in managing and operating the patients. YK assisted the pathological investigation. KY participated in collecting clinical data and images. TI participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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The Prognostic Impact of Cigarette Smoking on Patients with Non-small Cell Lung Cancer

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Introduction: The purposes of this study are to investigate the association between cigarette smoking and clinicopathological characteristics of patients with non-small cell lung cancer (NSCLC) and to evaluate its significance as a predictor of recurrence after resection.

Methods: A total of 2295 consecutive patients with NSCLC underwent complete resection with systematic node dissection between August 1992 and December 2006 at the National Cancer Center Hospital East.

Results: A statistically significant difference in the 5-year overall survival rate was observed between never and ever smokers in patients with stage I (92% and 76%, respectively, $p < 0.001$) NSCLC, whereas no difference was observed in stage II (57% and 52%, respectively, $p = 0.739$) and stage III (30% and 33%, respectively, $p = 0.897$). In patients with stage I NSCLC, 5-year recurrence-free proportions (RFPs) for never and ever smokers were 89% and 80%, respectively ($p < 0.001$). In contrast, the 5-year RFPs for never smokers were lower than those for ever smokers in stage II (44% and 60%, respectively, $p = 0.049$) and stage III (17% and 31%, respectively, $p = 0.004$). In stage I patients, significant difference in 5-year RFP was observed between never and ever smokers (89% and 83%, respectively) in patients with adenocarcinoma, but not in patients with nonadenocarcinoma (82% and 76%, respectively).

Conclusions: Smoking history showed different impact on postoperative recurrence in patients with NSCLC between stage I and stages II and III, and depending on histology in stage I patients. Disease stages should be considered while evaluating smoking history as a predictor of recurrence.

Key Words: Non-small cell lung cancer, Adenocarcinoma, Cigarette smoking, Thoracic surgery, Recurrence.

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Disclosure: The authors declare no conflicts of interest.

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Cigarette smoking is a well-known habitual risk factor for lung cancer[1] and is strongly associated with many other factors, such as low socioeconomic status,[2] poor nutrition,[3] comorbidity,[4] and impaired immune function.[5] These smoking-associated factors may contribute to poor survival of cigarette smokers after lung cancer resection. Although several studies have reported that cigarette smoking has a negative effect on lung cancer patient prognoses,[6–10] whether cigarette smoking affects the biological behavior of lung cancer and whether it can be a predictor of recurrence after resection remain unclear.

The purposes of this study are to investigate the association between cigarette smoking and clinicopathological characteristics of patients with non-small cell lung cancer (NSCLC) and to evaluate the significance of cigarette smoking as a predictor of recurrence after resection. To offset the prognostic impact of comorbidities associated with cigarette smoking, we investigated recurrence-free proportion (RFP) in addition to overall survival rate.

PATIENTS AND METHODS

Patients

Two thousand three hundred sixty-seven consecutive patients with NSCLC underwent complete resection with lobectomy or greater and systematic node dissection between August 1992 and December 2006 at our institution. Complete resection was defined as cancer-free surgical margins observed in both gross and histological examinations. Of these 2367 patients, 72 patients who underwent preoperative chemotherapy or radiation therapy, or both ($n = 43$) or had low-grade pulmonary malignancies ($n = 29$) including carcinoids, mucoepidermoid carcinomas, and adenoid cystic carcinomas were excluded from this study. The remaining 2295 patients were the subjects of this study.

Pathological Evaluations

Disease stages were diagnosed based on the tumor, node, metastasis (TNM) classification of the International Union Against Cancer, seventh edition.[11] Histological type of adenocarcinomas was determined according to the World Health Organization's classification.[12] Adenocarcinomas were histologically graded as well, moderately, or poorly differentiated carcinomas according to the degree of structural and cytological atypia. Bronchioloalveolar carcinoma (BAC) was categorized as a well-differentiated component,

acinar, and papillary adenocarcinomas as moderately differentiated components, and solid carcinoma with mucin production without any clear gland formation as a poorly differentiated component. When more than one differentiation component was identified in a tumor, the differentiation of the most predominant component was registered as its histological differentiation. Intratumoral vascular invasion (IVI) and visceral pleural invasion (VPI) were evaluated by hematoxylin and eosin and elastin (Victoria blue-van Gieson) staining. VPI was classified as defined in the TNM Classification, seventh edition.[11]

Patient Follow-Up

We examined patients at 3-month intervals for the first 2 years and at 6-month intervals thereafter on an outpatient basis. The follow-up evaluation included physical examination, chest radiography, and blood examination including that of pertinent tumor markers. Further evaluations, including computed tomography scans of the chest and abdomen, brain magnetic resonance imaging, and bone scintigraphy, were performed on the detection of any symptoms or signs of recurrence. Since 2004, integrated positron emission tomography and computed tomography have also been performed when appropriate.

We diagnosed recurrence on the basis of findings of physical examination and diagnostic imaging and confirmed the diagnosis histologically when clinically feasible. Date of recurrence was defined as the date of cytohistological proof. Nevertheless, in cases diagnosed on the basis of clinicoradiological findings, date of recurrence was defined as the date of identification by a physician.

Clinicopathological Information

We prospectively collected information on cigarette smoking status using the hospital outpatient clinic questionnaire, which was completed by patients at their first visit. We asked patients to record the age at which they started smoking, duration of smoking, and average daily cigarette consumption. No environmental cigarette smoke exposure data were collected. Smoking extent was quantified in pack-years (PY), with 1 PY equivalent to 20 cigarettes on average per day for 1 year. Before admission, all patients were required to stop smoking.

We reviewed each patient's medical record to obtain clinicopathological information, which included age (dichotomized at the median age of 65 years), gender, smoking extent (dichotomized at the median value of 43 PY in ever smokers), diameter of the tumor on resected specimens ($\leq$3 or >3 cm), tumor histology (adenocarcinoma or nonadenocarcinoma), tumor location (upper/middle lobe or lower lobe), tumor laterality (right or left), and pathological stage (stage I, II, or III based on the TNM classification, seventh edition).[11]

Statistical Analysis

Differences in categorical outcomes were evaluated by the χ^2 test. Continuous variables were compared using the t test. The length of overall survival rate was calculated in months from the date of resection to the date of death because of any cause or of last follow-up. The length of RFP was calculated in months from the date of resection to the date of the first recurrence or last follow-up. To calculate RFP, patients who died without recurrence or who were known to be recurrence free at the date of last contact were censored. In univariate analyses, all cumulative survival rates or RFPs were estimated using the Kaplan–Meier method, and differences in variables were evaluated using the log-rank test. Multivariate analyses were performed using the Cox proportional hazards regression model. All p values reported were two sided, and the significance level was set at less than 0.05. Analyses were performed using the statistical software SPSS version 11.0 (Dr. SPSS II for Windows, SPSS Inc., Chicago, IL) and GraphPad Prism (Prism for Windows, version 5.02, GraphPad Software Inc., La Jolla, CA).

Data collection and analyses were approved, and the need to obtain written informed consent from each patient was waived by the institutional review board in April 2010.

RESULTS

Smoking Extent and Clinicopathological Factors

The median follow-up period was 53 months (range, 1–163 months). The details of patient characteristics and smoking extent are shown in Table 1. Smoking extent was

TABLE 1. Patient Characteristics and Smoking Extent

Characteristics	No. of Patients (%)	Smoking Extent (PY ± SE)	p^a
Overall	2295	31.8 ± 0.7	
Age, yr (mean, 64.8; range, 20–89)			
≤65	1148 (50)	28.9 ± 0.9	
>65	1147 (50)	34.6 ± 1.0	<0.001
Gender			
Women	840 (37)	6.5 ± 0.5	
Men	1455 (63)	46.4 ± 0.8	<0.001
Tumor size (cm)			
≤3.0	1218 (53)	26.5 ± 0.9	
>3.0	1077 (47)	37.7 ± 1.0	<0.001
Tumor location			
Upper/middle lobe	1448 (63)	31.4 ± 0.8	
Lower lobe	847 (37)	32.3 ± 1.1	0.528
Tumor laterality			
Right	1383 (60)	32.2 ± 0.9	
Left	912 (40)	31.2 ± 1.0	0.463
Histological type			
Adenocarcinoma	1585 (69)	22.4 ± 0.7	
Nonadenocarcinoma	710 (31)	52.8 ± 1.1	<0.001
Stage			
I	1357 (59)	26.9 ± 0.8	
II	488 (21)	39.9 ± 1.4	<0.001[b]
III	450 (20)	37.5 ± 1.6	<0.001[b]

[a]By t test.
[b]Compared with stage I patients.
PY, pack-years; SE, standard error.

greater in older patients than in younger patients. Smoking was more common in male patients than in female patients. Smoking extent in patients with larger tumor size, nonadenocarcinoma, and stage II or higher tumors was significantly greater than those in patients with smaller tumor size, adenocarcinoma, or stage I tumors.

Correlation between Smoking Extent, Overall Survival Rates, and RFPs According to Stage

Patients were classified into the following three subgroups according to smoking extent: group 1, never smokers (PY = 0); group 2, 0 < PY ≤ 43; and group 3, PY > 43. Figures 1*A*, *B* show overall survival and RFP curves of patients stratified by smoking extent. Five-year overall survival rates of patients in groups 1 (PY = 0), 2 (0 < PY ≤ 43), and 3 (PY > 43) were 77.9%, 64.1%, and 57.0%, respectively. Statistically significant differences in survival rate were observed among each group, but the group 2 survival curve was apparently closer to the group 3 curve than to the group 1 curve. Five-year RFPs of patients in groups 1, 2, and 3 were 72.3%, 65.3%, and 65.3%, respectively. Statistically significant differences in RFPs were observed between groups 1 and 2 and between groups 1 and 3, whereas no difference was observed between groups 2 and 3. Therefore, patients in groups 2 and 3 were together defined as ever smokers (PY > 0; Figures 1*C*, *D*) and compared with never smokers (PY = 0) in the following analyses.

Figures 2*A–C* show the overall survival curves plotted according to the smoking history of patients with NSCLC in stages I, II, and III. A statistically significant difference in the 5-year overall survival rate was observed between never and ever smokers in patients with stage I (92.3% and 76.1%, Figure 2*A*) NSCLC, whereas no differences were observed in patients with stage II (57.0% and 51.7%, Figure 2*B*) and stage III (29.8% and 33.0%, Figure 2*C*) NSCLC.

Figures 2*D–F* show the RFP curves plotted according to the smoking history of patients with NSCLC in stages I, II, and III. In patients with stage I NSCLC, the 5-year RFP for never smokers (88.7%) was significantly higher than that for

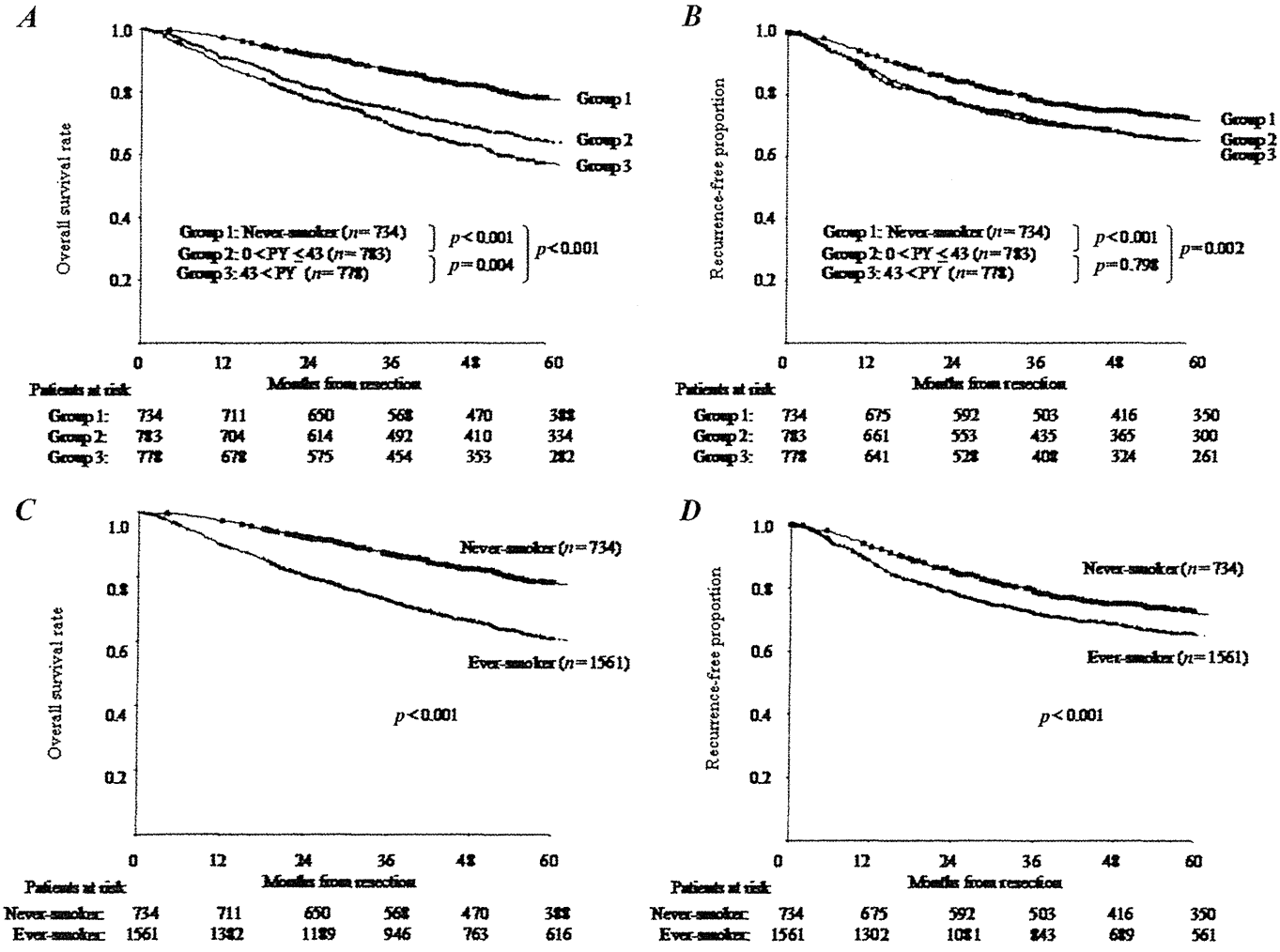

FIGURE 1. Overall survival and recurrence-free proportion (RFP) curves according to smoking status in the entire cohort. *A*, Overall survival curves according to smoking extent. *B*, RFP curves according to smoking extent. *C*, Overall survival curves according to smoking history. *D*, RFP curves according to smoking history. PY, pack-years.

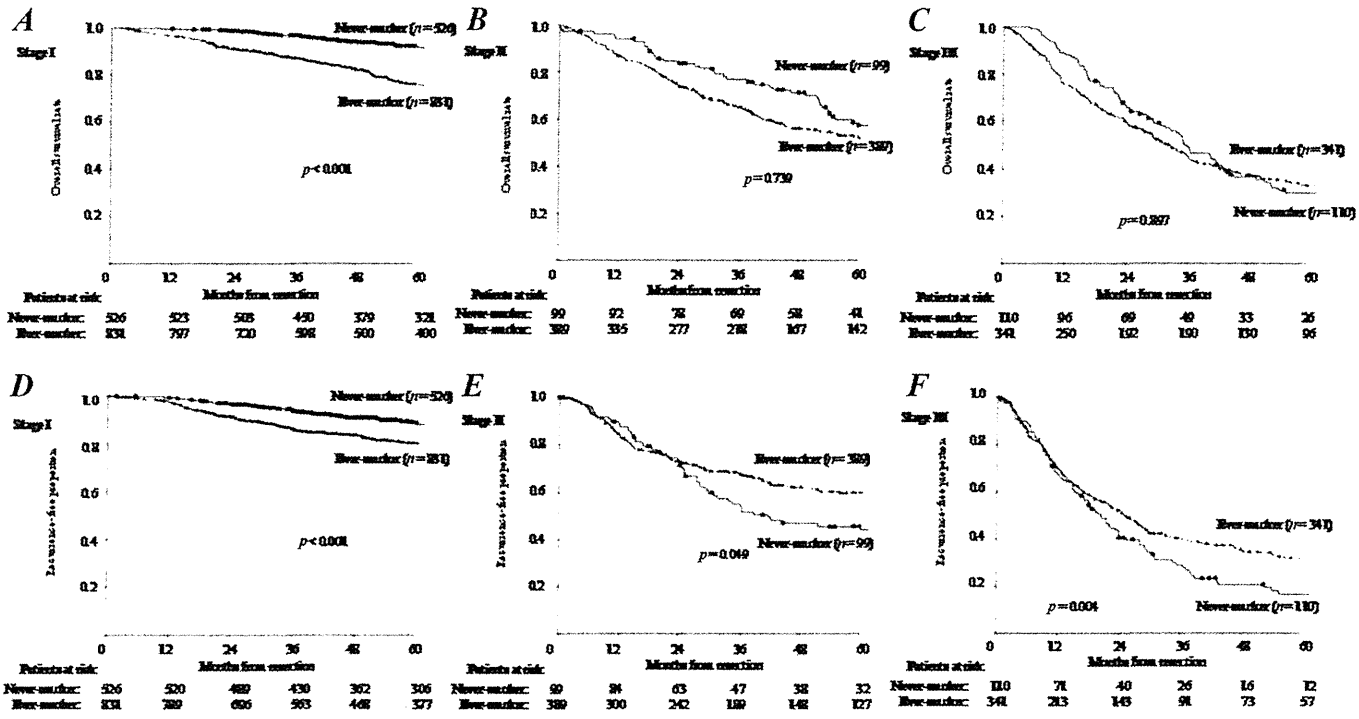

FIGURE 2. Overall survival and recurrence-free proportion (RFP) curves according to smoking history in each stage. *A*, Overall survival curves of patients with stage I non-small cell lung cancer (NSCLC). *B*, Overall survival curves of patients with stage II NSCLC. *C*, Overall survival curves of patients with stage III NSCLC. *D*, RFP curves of patients with stage I NSCLC. *E*, RFP curves of patients with stage II NSCLC. *F*, RFP curves of patients with stage III NSCLC.

ever smokers (80.3%). In contrast, the 5-year RFPs for never smokers were significantly lower than those for ever smokers in patients with stage II (44.2% and 59.8%, Figure 2*E*) and stage III (16.5% and 31.4%, Figure 2*F*) NSCLC.

Prognostic Impact of Cigarette Smoking on Patients with Stage I NSCLC

Table 2 lists 5-year overall survival rates and RFPs according to clinicopathological features of patients with stage I NSCLC. Univariate analysis identified the following five statistically significant prognostic and risk factors for recurrence: age, gender, smoking history, histology, and stage. In multivariate analysis, old age, ever smoking history, nonadenocarcinoma histology, and stage IB were found to be statistically significant independent unfavorable prognostic factors for overall survival (Table 3). Statistically significant independent risk factors for recurrence were ever smoking history and stage IB (Table 4).

Overall Survival Rates and RFPs for Never and Ever Smokers with Stage I NSCLC Stratified by Histological Type

Figures 3*A*, *B* show the overall survival curves of never and ever smokers with stage I NSCLC stratified by histological type. Among patients with stage I adenocarcinoma, 508 (49%) were never smokers and 523 (51%) were ever smokers. Patients with stage I nonadenocarcinoma included 18 (6%) never smokers and 308 (94%) ever smokers. Statistically significant differences in 5-year overall survival rates were observed between never and ever smokers in patients with adenocarcinoma (92.4% and 81.8%, respectively, Figure 3*A*) and patients with nonadenocarcinoma (88.2% and 66.8%, respectively, Figure 3*B*).

Figures 3*C*, *D* show the RFP curves of never and ever smokers with stage I NSCLC stratified by histological type. In patients with adenocarcinoma, a statistically significant difference in 5-year RFP was observed between never and ever smokers (88.9% and 82.7%, respectively, Figure 3*C*). No statistically significant difference was observed in patients with nonadenocarcinoma (82.2% and 76.3%, respectively, Figure 3*D*).

Correlation between Smoking History and Pathological Characteristics of Patients with Stage I Adenocarcinoma

To determine the reason for the RFP being significantly lower in ever smokers than in never smokers with stage I adenocarcinoma, we investigated pathological characteristics of patients with stage I adenocarcinoma. The correlation between smoking history and pathological characteristics of patients with stage I adenocarcinoma is shown in Table 5. Ever smokers showed significantly more moderately or poorly differentiated carcinomas and significantly more tumors with IVI or VPI than never smokers.

DISCUSSION

Several studies have shown the significance of cigarette smoking as a prognostic factor in patients with lung can-

TABLE 2. Univariate Analyses of Prognostic Factors in Patients with Stage I NSCLC

Characteristics	No. of Patients (%)	Overall Survival Rate at 5 yr (%)	Univariate *p* Value	Recurrence-Free Proportion at 5 yr (%)	Univariate *p* Value
Overall	1357	82.5		82.8	
Age (yr)					
≤65	678 (50)	89.3	<0.001[a]	86.3	0.002[a]
>65	679 (50)	75.4		79.8	
Gender					
Women	583 (43)	89.8	<0.001[a]	86.7	<0.001[a]
Men	774 (57)	76.9		79.8	
Smoking history					
Never smoker	526 (39)	92.3	<0.001[a]	87.7	<0.001[a]
Ever smoker	831 (61)	76.1		78.3	
Histological type					
Adenocarcinoma	1031 (76)	87.1	<0.001[a]	85.3	<0.001[a]
Nonadenocarcinoma	326 (24)	68		74.8	
Tumor location					
Upper/middle lobe	918 (68)	83.3	0.619	82.8	0.951
Lower lobe	439 (32)	80.8		82.9	
Tumor laterality					
Right	846 (62)	84	0.083	84.5	0.053
Left	511 (38)	80		80	
Stage					
IA	805 (59)	90.6	<0.001[a]	90.8	<0.001[a]
IB	552 (41)	70.6		72.9	

[a]Indicates significance.
NSCLC, non-small cell lung cancer.

TABLE 3. Multivariate Analyses of Prognostic Factors in Patients with Stage I NSCLC

Factors	Unfavorable	Favorable	HR	95% CI	*p*
Age (yr)	>65	<65	2.205	1.717–2.830	<0.001[a]
Gender	Men	Women	1.149	0.832–1.587	0.399
Smoking history	Ever smoker	Never smoker	1.833	1.273–2.640	0.001[a]
Histological type	Nonadenocarcinoma	Adenocarcinoma	1.513	1.179–1.943	0.001[a]
Stage	IB	IA	2.436	1.918–3.092	<0.001[a]

[a]Indicates significance.
HR, hazard ratio for death; CI, confidence interval; NSCLC, non-small cell lung cancer.

TABLE 4. Multivariate Analysis of Risk Factors for Recurrence in Patients with Stage I NSCLC

Factors	Unfavorable	Favorable	HR	95% CI	*p*
Age (yr)	>65	<65	1.205	0.928–1.564	0.161
Gender	Men	Women	1.038	0.732–1.472	0.835
Smoking history	Ever smoker	Never smoker	1.511	1.033–2.210	0.033[a]
Histological type	Nonadenocarcinoma	Adenocarcinoma	1.227	0.911–1.651	0.178
Stage	IB	IA	2.831	2.159–3.712	<0.001[a]

[a]Indicates significance.
HR, hazard ratio for recurrence; CI, confidence interval; NSCLC, non-small cell lung cancer.

cer.[6–10] A recent Japanese population-based study[6] reported that ever smokers showed an unfavorable postoperative prognosis compared with never smokers after complete NSCLC resection. However, cigarette smoking is also a well-known risk factor for severe pulmonary and cardiovascular diseases.[4] Several studies[8,13–15] found that approximately 20 to 40% of smokers with lung cancer died without evidence of cancer progression or recurrence. When patients who died of other

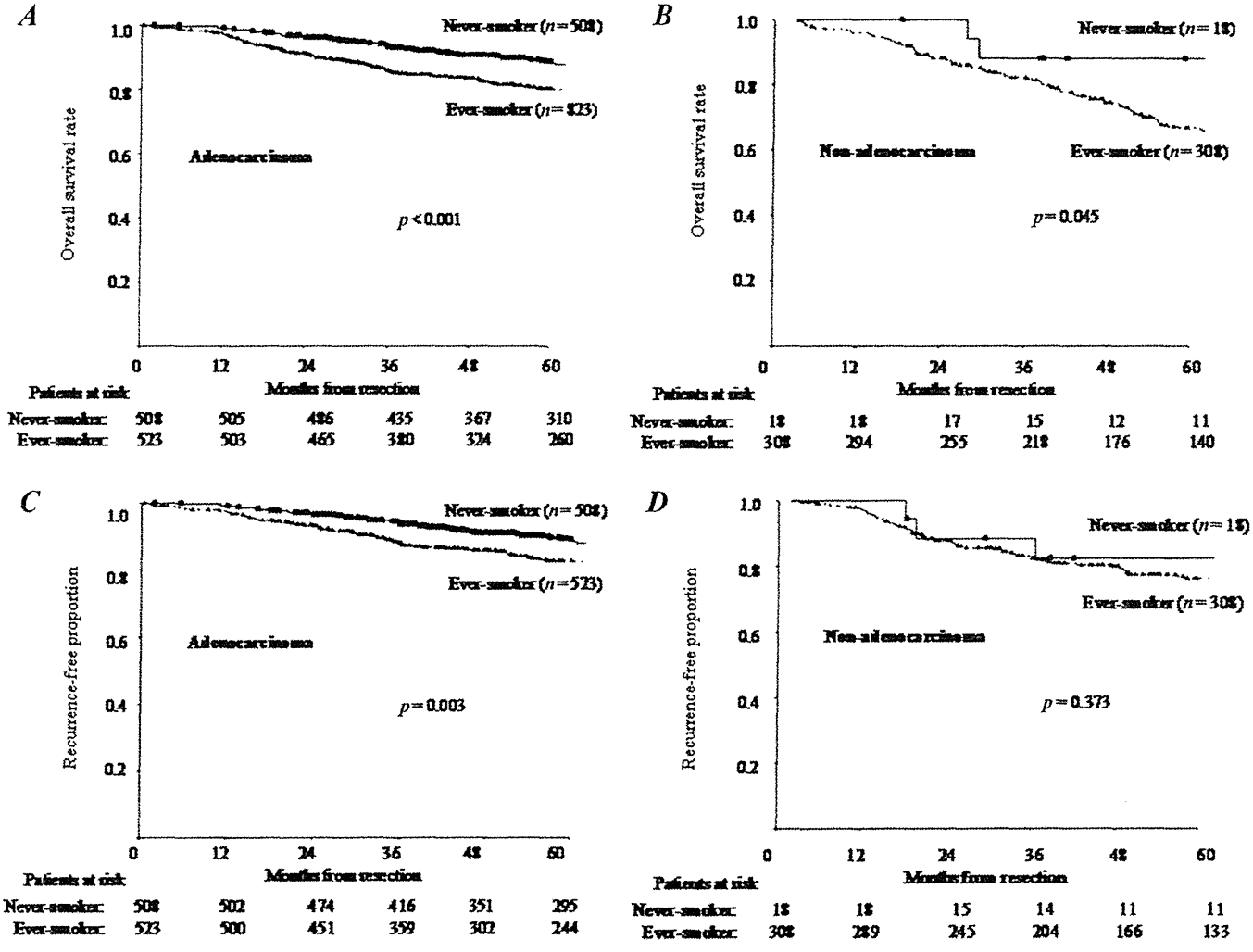

FIGURE 3. Overall survival and recurrence-free proportion (RFP) curves according to smoking history in patients with stage I non-small cell lung cancer. *A*, Overall survival curves of adenocarcinoma patients. *B*, Overall survival curves of nonadenocarcinoma patients. *C*, RFP curves of adenocarcinoma patients. *D*, RFP curves of nonadenocarcinoma patients.

diseases were excluded from the analysis, no significant differences in lung cancer-specific survival rates were reported to be observed between ever and never smokers.[6] Whether cigarette smoking causes significant biological aggressiveness in NSCLC, leading to more recurrence and metastasis after resection, remains unclear. In this study, we investigated the relationships between cigarette smoking and clinicopathological characteristics and evaluated the prognostic significance of cigarette smoking stratified by stage and histology.

We found that postoperative NSCLC recurrences were more frequent in ever smokers than in never smokers only in stage I patients. Some recent studies also reported that ever smoking history is an unfavorable prognostic factors in patients with stage I NSCLC.[16,17] Based on the results of multivariate analyses, ever smoking history, in addition to disease stage, was considered an independent postoperative predictor of recurrence in patients with stage I NSCLC. Brundage et al.[18] found 169 prognostic factors for patients with NSCLC reported in 887 studies published between 1990 and 2001. Although most of these factors are not readily observed in routine clinical practice, cigarette smoking history is the most commonly observed factor.

When we evaluated the prognostic significance of cigarette smoking stratified by histology among patients with stage I NSCLC, significant differences in both overall survival and RFP were observed between never and ever smokers in patients with adenocarcinoma. In patients with nonadenocarcinoma, however, significant differences were observed in overall survival but not in RFP, which might be attributable to the small number of stage I nonadenocarcinoma never smokers. This result suggests that stage I adenocarcinomas in ever smokers are more aggressive than those in never smokers. Pathological characteristics of stage I adenocarcinomas showed that tumors in ever smokers were significantly more frequently poorly differentiated and were accompanied by IVI or VPI than those in never smokers. These aggressive and invasive characteristics might be the reason for ever smokers

TABLE 5. Correlation between Smoking History and Pathological Characteristics of Patients with Stage I Adenocarcinoma

Characteristics	Smoking History, No. of Patients (%)		
	Never Smoker	Ever Smoker	p^a
Total	508 (49)	523 (51)	
Histological differentiation			
Well differentiated	301 (59)	179 (34)	<0.001[b]
Moderately/poorly differentiated	207 (41)	344 (66)	
Lymphatic permeation			
Absent	423 (83)	430 (82)	0.681
Present	85 (17)	93 (18)	
Intratumoral vascular invasion			
Absent	419 (82)	333 (64)	<0.001[b]
Present	89 (18)	190 (36)	
Pleural invasion			
Absent	422 (83)	407 (78)	0.034[b]
Present	86 (17)	116 (22)	

[a] χ^2 test.
[b] Indicates significance.

developing more frequent recurrence than never smokers among patients with stage I adenocarcinoma.

Cigarette smoke is known to contain numerous mutagenic and carcinogenetic chemicals that may cause mutations in tumor suppressor genes such as *p53* and in oncogenes such as K-*ras*.[19–21] Suzuki et al.[20] reported that tumors with *p53* gene alterations showed high growth fraction percentages. Tollerud et al.[21] reported that cigarette smoking reduces local airway immunity, and alveolar macrophages activated by smoking suppress natural killer cell activity by producing prostaglandins and oxygen radicals. These findings may explain the aggressive and invasive nature of stage I adenocarcinomas in ever smokers. In addition, many biomarkers have been shown to be prognostic indicators of NSCLC, including serum carcinoembryonic antigen, *erbB2/Neu*, *BclII*, promoter hypermthylation of *hMSH2* mismatch repair gene, and overexpression of circulating c-met.[22–26] In addition to pathological factors, correlation between these biomarkers and smoking-related adenocarcinoma needs to be examined in the future study.

Guo et al.[16] and Bryant and Cerfolio[17] reported no significant statistical differences in overall survival rate between never smokers and ever smokers with stage II and stage III NSCLC. In this study, significantly lower RFPs were observed in never smokers than in ever smokers with stage II and III NSCLC, although no significant differences were observed in overall survival. These findings suggest that the significance of smoking history in postoperative outcome differs according to disease stage, and disease stages should be considered while evaluating smoking history as a predictor of recurrence after resection. However, we could not fully explain the reason for the opposite results of the significance of smoking history as a predictor of recurrence according to stage. Bryant and Cerfolio[17] reported that in patients with NSCLC, never smokers had more poorly differentiated tumors with higher maximum standardized uptake value of ^{18}F-fluorodeoxyglucose on positron emission tomography scans compared with ever smokers. The ^{18}F-fluorodeoxyglucose uptake correlates with the proliferative activity of tumors and is reported to be an independent prognostic factor in patients with lung cancer.[27,28] Among patients with adenocarcinoma, the number of never smokers with BAC subtype has recently increased in Japan, and BAC is often found at an earlier stage and reported to be associated with a favorable prognosis.[29–31] Therefore, one possible explanation would be that cancer histologic type distribution is different between never and ever smokers and that the distribution is also different between stages.

This retrospective study had several limitations in the analyses. In particular, smoking status was reported by patients and was not confirmed biochemically, and therefore, the data may be biased. Ethnic diversity was lacking in our 100% Japanese patient population. Second-hand tobacco smoke is an established cause of lung cancer, but it was too difficult to quantify this factor objectively and include it in the analyses. Another limitation is that because nonadenocarcinoma never smokers were a mere fraction of the entire cohort, we could not fully examine the correlation between cigarette smoking and nonadenocarcinoma. Despite these limitations, our results showed the stage in which cigarette smoking had a prognostic impact after complete NSCLC resection.

CONCLUSION

Smoking history showed different impact on postoperative recurrence in NSCLC patients between stage I and stages II and III, and depending on histology in stage I patients. Disease stages and histology should be considered while evaluating smoking history as a predictor of recurrence after resection.

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Influence of Cigarette Smoking on Histological Subtypes of Stage I Lung Adenocarcinoma

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Background: The purpose of this study was to examine the association between cigarette smoking and histological subtypes of lung adenocarcinoma.

Methods: We reviewed a total of 320 consecutive patients with stage I adenocarcinoma who underwent complete resections with systematic node dissections from January 2004 to December 2006 at the National Cancer Center Hospital East.

Results: A statistically significant difference was observed in recurrence-free probabilities between never smokers and ever smokers (3-year recurrence-free probabilities of 95.6% and 88.6%, respectively, $p = 0.034$). Among adenocarcinoma histological subtypes, only a solid component was significantly more frequent in ever smokers than in never smokers ($p < 0.001$). Among patients with solid components, significantly more cases had lymphatic permeation ($p = 0.007$), intratumoral vascular invasion ($p < 0.001$), and visceral pleural invasion ($p < 0.001$). Multivariate analysis revealed that ever-smoking history was the only statistically significant independent clinical predictor for a solid component ($p < 0.001$). Among ever smokers, smoking extent in pack-years of patients with solid components was significantly greater than that of those without solid components ($p < 0.001$). With respect to predominant subtypes, smoking extent in pack-years of patients with predominantly solid adenocarcinomas was significantly greater than that of patients with predominantly bronchioloalveolar carcinoma, papillary, or acinar adenocarcinomas (all $p < 0.001$).

Conclusion: A greater smoking extent was associated with the presence of adenocarcinoma solid components, which may have more aggressive biological features resulting in poorer outcomes.

Key Words: Lung cancer, Adenocarcinoma, Subtype, Thoracic surgery, Cigarette smoking, Solid component.

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Adenocarcinoma of the lung is the most frequent histological subtype of lung cancer, and its incidence is increasing in most countries.[1] In Japan, adenocarcinoma is also the most common histological subtype of resected lung cancers, accounting for more than 60% of cases.[2]

Adenocarcinomas are typically very heterogeneous, showing a wide variety of histological features, including bronchioloalveolar carcinoma (BAC), acinar, papillary, and solid adenocarcinoma.[3] Among these major histological subtypes, BAC is often reported to be associated with a favorable prognosis,[4–6] whereas the other subtypes are considered invasive components and are associated with poor outcomes, particularly solid components.[7,8]

Smoking is a well-known causative factor of lung cancer[9] and is associated with all the histological subtypes of lung cancer.[10–12] Although the association of cigarette smoking with adenocarcinoma is reported to be the weakest,[12] its association with carcinogenesis of lung adenocarcinoma is established. Several studies have recently reported that ever smokers had significantly unfavorable prognoses compared with never smokers among patients with lung adenocarcinoma.[13,14] Because the association between smoking and postoperative complications is well known,[13,15] this factor may partially contribute to unfavorable cancer survivals of ever smokers. Another possible reason is that the number of patients with BAC subtypes among never smokers has recently increased,[16] which may also partially contribute to the favorable prognoses among never smokers.

Although many studies have reported on the associations between cigarette smoking and lung adenocarcinomas,[13,14] several questions regarding the influence of cigarette smoking on lung adenocarcinomas remain unanswered. Primarily, whether cigarette smoking affects the biological behaviors of lung adenocarcinomas, especially histological subtypes of adenocarcinoma? If so, with which subtype(s) is cigarette smoking associated? To answer these questions, we reviewed a series of consecutive patients with pathological stage I adenocarcinomas who underwent complete resections in our hospital. The main purpose of this study was to investigate the association between cigarette smoking and the histological subtypes of adenocarcinoma.

PATIENTS AND METHODS

Patients Selection

A total of 466 consecutive patients with clinical stage I adenocarcinoma underwent operation from January 2004 to

December 2006 at the National Cancer Center Hospital East. We excluded three patients from our study because they had received preoperative chemotherapy, radiation therapy, or both. Among the 463 patients, 458 patients underwent complete surgical resection. The operative findings and pathological examination of surgical specimens revealed that 90 patients were reclassified as pathological stage II or higher and were up-staged. Among the 368 patients diagnosed as pathological stage I, 48 patients underwent limited surgery. The remaining 320 patients with pathological stage I adenocarcinoma who underwent complete tumor resection with lobectomy or a more extensive surgery along with systematic lymph node dissection were enrolled as the subjects of this study.

Pathological Evaluations

Disease stages were diagnosed based on the TNM classification of the International Union Against Cancer, 7th edition.[17] The histological type was determined according to the World Health Organization's classification.[3] Intratumoral vascular invasion (IVI) and visceral pleural invasion (VPI) were evaluated by staining with hematoxylin-eosin and Victoria blue-van Gieson stains. VPI was classified according to the TNM classification, 7th edition.[17] Adenocarcinoma histological subtypes were categorized into BAC (nonmucinous or mucinous), papillary, acinar, and solid adenocarcinomas according to the World Health Organization's classification.[3] Mucin production in a solid adenocarcinoma component was confirmed by the alcian blue-periodic acid Schiff method. We determined the predominant subtype, and each component was defined as present if observed in more than 1 of 10 of a tumor; otherwise, it was defined as absent.

Clinicopathological Information

We prospectively collected information on cigarette smoking status using outpatient clinic questionnaires, which were answered by patients on their first clinic visit. Patients were asked to record the age when they started smoking, duration of smoking, and average daily cigarette consumption. No environmental cigarette smoke exposure data were collected. The extent of smoking was quantified in pack-years (PY), with 1 PY equivalent to 20 cigarettes, on average, per day for 1 year. Before admission, all patients were required to stop smoking.

We reviewed the medical records of each patient for clinicopathological information. This included age (dichotomized at the median age of 65 years), gender, smoking history (never- or ever smoker), smoking extent in PY, forced expiratory volume in 1 second % (<70% or ≥70%), preoperative serum carcinoembryonic antigen (CEA) level (cutoff at the normal upper limit of 5 ng/ml), tumor laterality (right or left), primary lobe (upper, middle, or lower lobe), tumor size (≤3 cm or >3 cm), BAC component (present or absent), papillary component (present or absent), acinar component (present or absent), solid component (present or absent), predominant histological subtypes (BAC, papillary, acinar, or solid), lymphatic permeation (present or absent), IVI (present or absent), and VPI (as defined in the TNM classification, 7th edition,[17] present or absent).

Statistical Analysis

Differences in categorical outcomes were evaluated by χ^2 test. Continuous variables were compared using t tests. To offset the prognostic impact of comorbidities associated with cigarette smoking, we investigated recurrence-free probabilities for this study. The length of recurrence-free probability was calculated in months from the date of resection to the date of first recurrence or last follow-up. To calculate the recurrence-free probability, patients who died without recurrence or who were known to be recurrence free at the date of last contact were excluded from the calculation. For univariate analyses, all recurrence-free probabilities were estimated using the Kaplan–Meier method, and comparisons of these variables were made using the log-rank test. Multivariate analyses were performed using Cox's proportional hazard regression model. Clinical predictors for the presence of a solid component were evaluated by logistic regression analyses. The predictors from univariate analyses were also evaluated using multiple regression analyses. The p value less than 0.2 in a univariate model was set as the threshold used for selection of variables in a multivariate model. All reported p values were two sided, and the significance level 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) and GraphPad Prism (Prism for Windows, Version 5.02, GraphPad Software, Inc., La Jolla, CA).

Data collection and analyses were approved, and the need to obtain written informed consent from each patient was waived by the institutional review board in April 2010.

RESULTS

Patient Characteristics and Recurrence-Free Probabilities According to Clinicopathological Factors

The median follow-up period was 37 months (range: 3–60 months). Recurrence-free probabilities according to clinicopathological factors are presented in Table 1. Univariate analysis (log-rank test) identified eight significant risk factors for recurrence: gender, smoking history, preoperative serum CEA level, tumor diameter, the presence of solid component, lymphatic permeation, IVI, and VPI (Table 1).

A statistically significant difference was observed in recurrence-free probabilities between never smokers and ever smokers: 3-year recurrence-free probabilities of 95.6% and 88.6%, respectively (Figure 1). Figures 2*A–D* show the recurrence-free probability curves according to the histological subtypes. No statistically significant differences were present in the recurrence-free probabilities between patients with and without BAC (3-year recurrence-free probabilities of 90.2% and 92.9%, respectively; $p = 0.522$; Figure 2*A*), with and without papillary (88.8% and 93.2%, respectively; $p = 0.539$; Figure 2*B*), and with and without acinar (95.2% and 88.9%, respectively; $p = 0.092$; Figure 2*C*) components. In contrast, the 3-year recurrence-free probability for patients with solid components (84.9%) was significantly lower than that for those without solid components (96.3%; $p = 0.001$; Figure 2*D*).

TABLE 1. Patient Characteristics, and Recurrence-Free Probabilities According to Clinicopathological Factors

Characteristics	No. of Patients (%)	Recurrence-Free Probability 3-year (%)	Univariate *p* Value†	Multivariate Analysis HR	95% CI	*P*-Value
Total	320	92.2				
Clinical factors						
Age (years)						
≤65	169 (53)	93.5	0.534	Not included multivariable model		
>65	151 (47)	90.5				
Gender						
Women	175 (55)	95.8	0.009*	1		
Men	145 (45)	87.4		1.282	0.330–4.986	0.72
Smoking history						
Never-smoker	148 (46)	95.6	0.034*	1		
Ever-smoker	172 (54)	88.6		1.644	0.354–7.629	0.525
CEA						
Within normal range	148 (46)	94.9	0.007*	1		
Elevated	172 (54)	85		1.221	0.499–2.989	0.661
FEV1 %						
≥70	277 (87)	92.8	0.591	Not included multivariable model		
<70	42 (13)	87.9				
Tumor laterality						
Right	206 (64)	93.5	0.499	Not included multivariable model		
Left	114 (36)	90				
Primary lobe						
Upper or middle lobe	227 (71)	93.3	0.297	Not included multivariable model		
Lower lobe	93 (29)	89.4				
Tumor size (cm)						
≤3.0	253 (79)	96.4	0.015*	1		
>3.0	67 (21)	89.5		1.165	0.393–3.457	0.783
Histological subtypes						
BAC component						
Absent	95 (30)	90.2	0.522	Not included multivariable model		
Present	225 (70)	92.9				
Papillary component						
Absent	83 (26)	88.8	0.539	Not included multivariable model		
Present	237 (74)	93.2				
Acinar component						
Absent	163 (51)	95.2	0.092	1		
Present	157 (49)	88.9		1.151	0.474–2.797	0.756
Solid component						
Absent	199 (62)	96.3	0.001*	1		
Present	121 (38)	84.9		1.59	0.661–3.823	0.301
Pathological factors						
Lymphatic permeation						
Absent	297 (93)	94.8	<0.001*	1		
Present	23 (7)	58.4		2.698	1.050–6.932	0.039*
Intratumoral vascular invasion						
Absent	245 (77)	98.6	<0.001*	1		
Present	75 (23)	70.9		14.65	3.804–56.422	<0.001*
Visceral pleural invasion						
Absent	261 (82)	95.5	<0.001*	1		
Present	59 (18)	77.9		1.006	0.393–2.581	0.989
Gene mutation status						
EGFR						
Wild type	71 (22)	87.7	0.783	Not included multivariable model		
Mutated	12 (4)	90				
Not examined	237					

*, significance; Numbers in parentheses, percentages; †, log-rank test; HR, hazard ratio; CI, confidence interval; CEA, preoperative serum carcinoembryonic antigen level, normal upper limit at 5 ng/mL; FEV1%, forced expiratory volume % in one second; BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor.

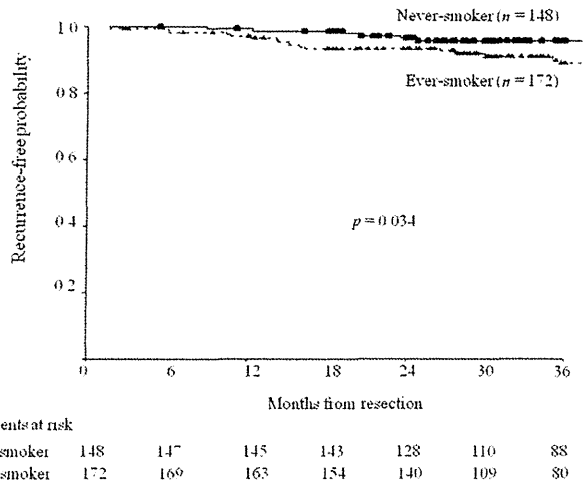

FIGURE 1. Recurrence-free probability curves according to smoking history in the entire cohort.

On multivariate analysis using the Cox regression model, presence of lymphatic permeation and presence of IVI remained statistically significant independent predictors for postoperative recurrence after resection (Table 1).

Univariate and Multivariate Analyses of Clinical Predictors for the Presence of a Solid Component

Because of the significantly lower recurrence-free probability only for patients with solid components among adenocarcinoma histological subtypes, we examined clinical predictors for the presence of a solid component. Univariate analyses revealed three significant clinical predictors for the presence of a solid component: gender, smoking history, and CEA (Table 2). On multivariate analysis, ever-smoking history was the only statistically significant independent clinical predictor for the presence of a solid component ($p < 0.001$; Table 2).

Associations between Solid Components and Other Pathological Factors

To clarify the reasons why patients with solid components had the significantly lower recurrence-free probability compared with those without solid components, we examined

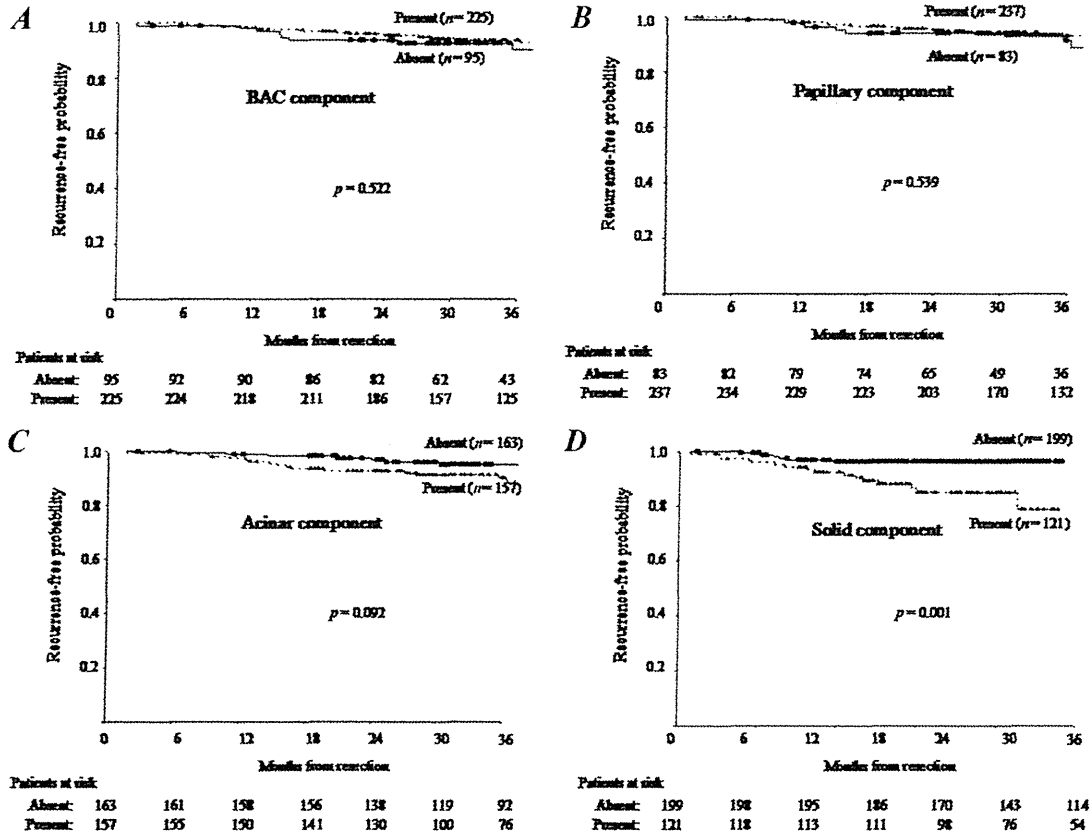

FIGURE 2. Recurrence-free probability curves according to the histological subtypes in the entire cohort. *A*, Recurrence-free probability curves of patients with and without bronchioloalveolar carcinoma (BAC) components. *B*, Recurrence-free probability curves of patients with and without papillary components. *C*, Recurrence-free probability curves of patients with and without acinar components. *D*, Recurrence-free probability curves of patients with and without solid components.

TABLE 2. Univariate and Multivariate Analyses of Predictors for the Presence of a Solid Component

	Univariate Analysis			Multivariate Analysis		
Variables	**HR**	**95% CI**	p^a	**HR**	**95% CI**	p^b
Age (yr)						
>65	1.37	0.871–2.156	0.173	1.423	0.859–2.359	0.642
≤65	1			1		
Gender						
Male	3.182	1.988–5.092	<0.001[c]	1.543	0.852–2.795	0.152
Female	1			1		
Smoking habits						
Ever smoker	4.419	2.680–7.287	<0.001[c]	3.319	1.779–6.191	<0.001[c]
Never smoker	1			1		
CEA						
Elevated	2.103	1.222–3.316	<0.001[c]	1.517	0.872–2.639	0.14
Within normal range	1			1		
FEV_1%						
<70	1.798	0.936–3.454	0.078	1.032	0.495–2.155	0.933
≥70	1			1		
Tumor laterality						
Right	1.182	0.739–1.890	0.486	Not included in multivariable model		
Left	1					
Primary lobe						
Upper or middle lobe	1.079	0.654–1.776	0.767	Not included in multivariable model		
Lower lobe	1					
Tumor size (cm)						
>3.0	1.443	0.836–2.491	0.187	1.156	0.629–2.124	0.742
≤3.0	1			1		

[a] Logistic regression procedure.
[b] Multiple regression analysis.
[c] Significance.
CEA, preoperative serum carcinoembryonic antigen level, normal upper limit at 5 ng/ml; FEV_1%, forced expiratory volume % in 1 second; HR, hazard ratio; CI, confidence interval.

associations between solid components and pathological factors including statistically significant independent predictors for recurrence such as lymphatic permeation and IVI (Table 3). Among patients with solid components, significantly more cases were found with lymphatic permeation ($p = 0.007$), IVI ($p < 0.001$), and VPI ($p < 0.001$). In addition, significantly more cases with epidermal growth factor receptor (EGFR) mutations were found in patients without solid components ($p = 0.033$).

Associations between Smoking History or Smoking Extent and Adenocarcinoma Histological Subtypes

The presence of a solid component was strongly associated with cigarette smoking (Table 2). Further evaluations were performed for elucidating the association between cigarette smoking and histological subtypes other than solid components in addition to other pathological factors (Table 4).

A BAC or papillary component was significantly more frequent in never smokers than in ever smokers ($p < 0.001$ and $p = 0.01$, respectively; Table 4). Among ever smokers, significantly more cases were found with IVI ($p = 0.025$). In addition, significantly more cases with EGFR mutations were found in never smokers ($p = 0.001$).

Figures 3*A–D* show the associations between smoking extent and histological subtypes for ever smokers only. In ever smokers only, the smoking extent in PY of patients with BAC components (mean = 25.0 ± 2.5) was significantly lower than that of those without BAC components (mean = 44.9 ± 3.8; Figure 3*A*). In contrast, the smoking extent in PY of patients with solid components (mean = 45.2 ± 3.2) was significantly greater than for those without solid components (mean = 30.0 ± 2.7; $p < 0.001$; Figure 3*D*).

Figure 4 shows the smoking extent in PY of all patients stratified by their predominant histological subtypes. The smoking extent in PY of patients with predominantly solid adenocarcinomas (PY = 45.5 ± 4.5) was significantly greater than that of those with predominantly BAC (PY = 14.5 ± 2.2), papillary (PY = 16.6 ± 2.4), or acinar (PY = 23.5 ± 4.7) adenocarcinomas (all $p < 0.001$).

Figure 5 shows the association between smoking extent and the proportions of tumors with solid components in the entire study cohort. Patients with higher proportions of solid tumor components also had greater smoking extent in PY. The smoking extent in PY of patients who had tumors with more than 50% solid components was greater than that of patients who had tumors with 10 to 50% solid components

TABLE 3. Associations between Solid Components and Clinicopathological Factors

Characteristics	No. of Patients (%)	Solid Component		p^a
		Absent	Present	
Total	320	199	121	
Pathological factors				
Lymphatic permeation				
Absent	297	191 (64)	106 (36)	0.007[b]
Present	23	8 (35)	15 (65)	
Intratumoral vascular invasion				
Absent	245	171 (70)	74 (30)	<0.001[b]
Present	75	28 (37)	47(63)	
Visceral pleural invasion				
Absent	261	176 (67)	85 (33)	<0.001[b]
Present	59	23 (39)	36 (61)	
Gene mutation status				
EGFR				
Wild type	71	35 (49)	36 (51)	0.033[b]
Mutated	12	10 (83)	2 (17)	
Not examined	237			

Numbers in parentheses represent percentages
[a] χ^2 test
[b] Significance.
EGFR, epidermal growth factor receptor.

TABLE 4. Associations between Smoking History and Adenocarcinoma Histological Subtypes

Characteristics	No. of Patients	Smoking Status		p^a
		Never Smoker	Ever Smoker	
Total	320	148	172	
Histological subtypes				
BAC component				
Absent	95	23 (24)	72 (76)	<0.001[b]
Present	225	125 (56)	100 (44)	
Papillary component				
Absent	83	28 (34)	55 (66)	0.01[b]
Present	237	120 (51)	117 (49)	
Acinar component				
Absent	163	74 (45)	89 (55)	0.823
Present	157	74 (47)	83 (53)	
Solid component				
Absent	199	118 (59)	81 (41)	<0.001[b]
Present	121	30 (25)	91 (75)	
Pathological factors				
Lymphatic permeation				
Absent	297	138 (46)	159 (54)	0.831
Present	23	10 (43)	13 (57)	
Intratumoral vascular invasion				
Absent	245	122 (50)	123 (50)	0.025[b]
Present	75	26 (35)	49 (65)	
Visceral pleural invasion				
Absent	261	123 (47)	138 (53)	0.564
Present	59	25 (42)	34 (58)	
Gene mutation status				
EGFR				
Wild type	71	22 (31)	49 (69)	0.001[b]
Mutated	12	10 (83)	2 (17)	
Not examined	237			

Numbers in parentheses represent percentages.
[a] χ^2 test.
[b] Significance.
BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor.

($p < 0.001$) and patients who had tumors without solid components ($p < 0.001$).

DISCUSSION

Cigarette smoking is a well-known risk factor for lung carcinogenesis.[9] Recent studies have indicated that ever smokers show significantly unfavorable prognoses, when compared with never smokers, particularly those with adenocarcinomas.[13,14] It has been suggested that cigarette smoking is associated with not only lung carcinogenesis but also unfavorable prognoses for lung adenocarcinomas. One possible reason why smokers with lung adenocarcinomas had more unfavorable prognoses than never smokers in previous reports[13] is that cigarette smoking is strongly associated with factors such as low socioeconomic status,[18] poor nutrition,[19] comorbidities,[20] and impaired immune function.[21] These smoking-associated factors may contribute to poor survival rates of cigarette smokers after lung cancer resections. Nevertheless, in this study, ever smokers had a significantly lower recurrence-free probability compared with never smokers with stage I adenocarcinomas, which suggests that smoking-related lung adenocarcinomas may behave more aggressively or result in unfavorable survival, regardless of cigarette smoking-related comorbidities.

The histological subtypes of adenocarcinoma mainly comprised BAC, acinar, papillary, and solid components.[3] Adenocarcinomas are histologically very heterogeneous, with only a minority of cases showing pure histological patterns.[3] Among these components, a solid component is the most poorly differentiated subtype.[22,23] Several studies have reported that the presence of a solid component is indicative of tumor invasiveness, proliferation, and dedifferentiation.[22,23] Riquet et al.[22] reported that patients with solid components have significantly poorer outcomes compared with those without solid components among patients with stage I adenocarcinoma. Also in this study, the 3-year recurrence-free probability for patients with solid components was significantly lower than that for patients without solid components.

In this study, the presence of a solid component was more frequently associated with an invasive or aggressive pathologic status, including lymphatic permeation, IVI, and VPI. This indicates that the presence of a solid component induces a more invasive and aggressive nature of lung adenocarcinomas, which is reflected by worse outcomes. There may be several possible explanations for the more aggressive and invasive biological characteristics of solid components observed in this study. Ding et al.[24] reported that mutations in the *p53* gene were negatively correlated with acinar, papillary, and BAC subtypes but were significantly positively