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

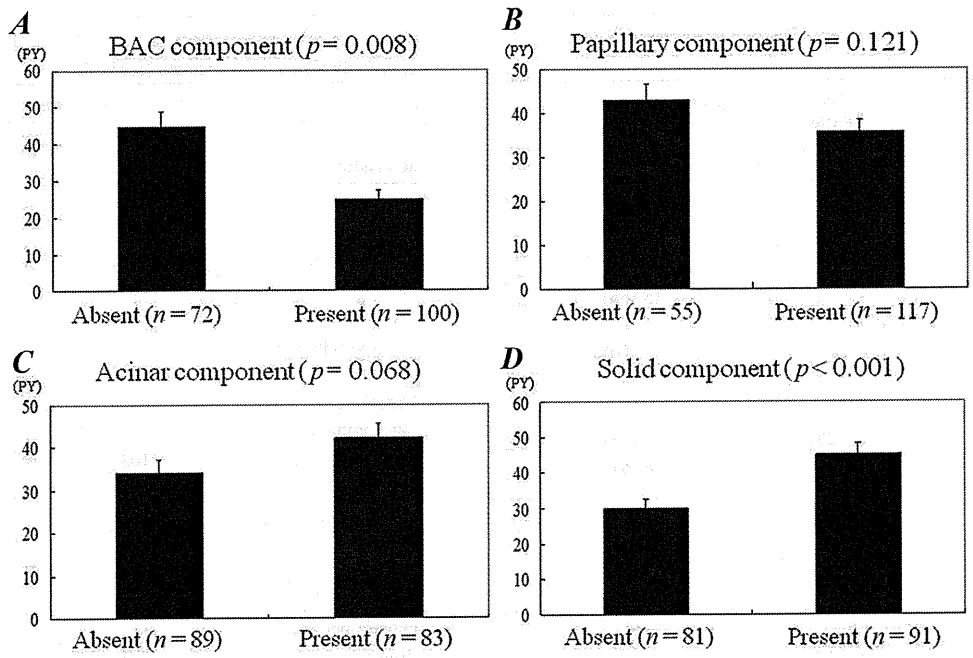

FIGURE 3. Associations between smoking extent and histological subtypes among ever smokers only. *A*, Bronchioloalveolar carcinoma (BAC) components. *B*, Papillary components. *C*, Acinar components. *D*, Solid components.

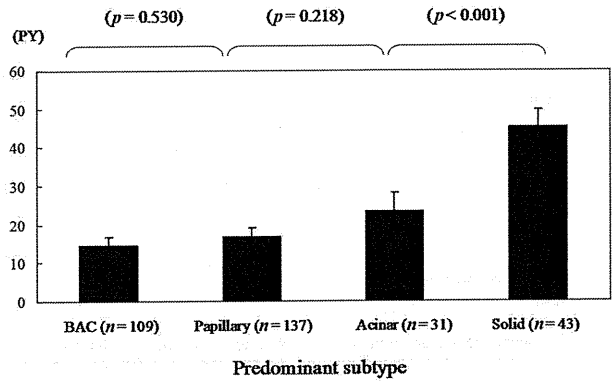

FIGURE 4. Smoking extent in pack-years (PY) of patients stratified according to predominant histological subtypes.

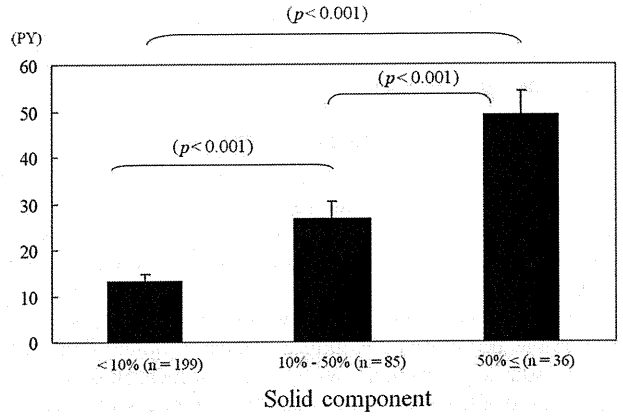

FIGURE 5. Association between smoking extent and the proportions of solid tumor components in the entire cohort.

correlated with solid subtypes of pulmonary adenocarcinomas. Suzuki et al.[25] reported that tumors with *p53* gene alterations showed higher growth fraction percentages, which may be the reason why a solid component was more aggressive and invasive and resulted in worse outcomes.

Suzuki et al.[25] also reported that a p53 mutation in lung cancer is closely associated with lifetime cigarette consumption. In this study, a solid component was more likely to be present in ever smokers than in never smokers. When evaluating ever smokers only, the smoking extent in PY of patients with solid components was significantly greater than that of those without solid components, which demonstrated that the presence of a solid component was strongly associated with a greater smoking extent.

In this study, ever-smoking history was a strong clinical predictor for the presence of a solid component. Several researchers have recently reported successful results with limited surgical resections for small adenocarcinomas.[26–30] Nevertheless, locoregional recurrence after limited resection is not rare, even in patients with a pathologically confirmed negative surgical margin.[31] This is probably due to intratumoral vessel involvement.[32] The presence of a solid component was significantly associated with histologically invasive characteristics such as lymphatic permeation, IVI and VPI, and it may be prudent to avoid limited surgery for patients with these invasive components. Therefore, we should be careful when proposing limited surgery for patients with

adenocarcinoma with smoking histories, particularly those with greater smoking extent.

In this study, significantly more cases with EGFR mutations were found in never smokers and patients without solid components. Ding et al.[24] reported that an EGFR mutation showed significant positive correlations with BAC and papillary subtypes but not with the solid subtype. In this study, a BAC or papillary component was significantly more frequent in never smokers than in ever smokers. In contrast, significantly more patients with solid components were found among ever smokers. The associations between adenocarcinoma histological subtypes and cigarette smoking demonstrated by our study may partly explain why EGFR mutations tended to be more frequent in the adenocarcinomas of never smokers than those of ever smokers in previous studies.[33]

This was a retrospective study, and the analyses conducted had several limitations. In particular, smoking extent was reported by the patients but was not confirmed biochemically and, therefore, may be biased. Objective quantification of environmental cigarette smoke exposure was difficult and was, therefore, not included in the analyses. Another limitation was a lack of ethnic diversity in our 100% Japanese patient population. Despite these limitations, we have clearly shown the influence of cigarette smoking on lung adenocarcinomas, particularly the associations between cigarette smoking and adenocarcinoma histological subtypes.

CONCLUSION

A greater smoking extent is associated with the presence of a solid tumor component, which may have more aggressive biological features and result in poorer outcomes.

ACKNOWLEDGMENTS

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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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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 (≤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

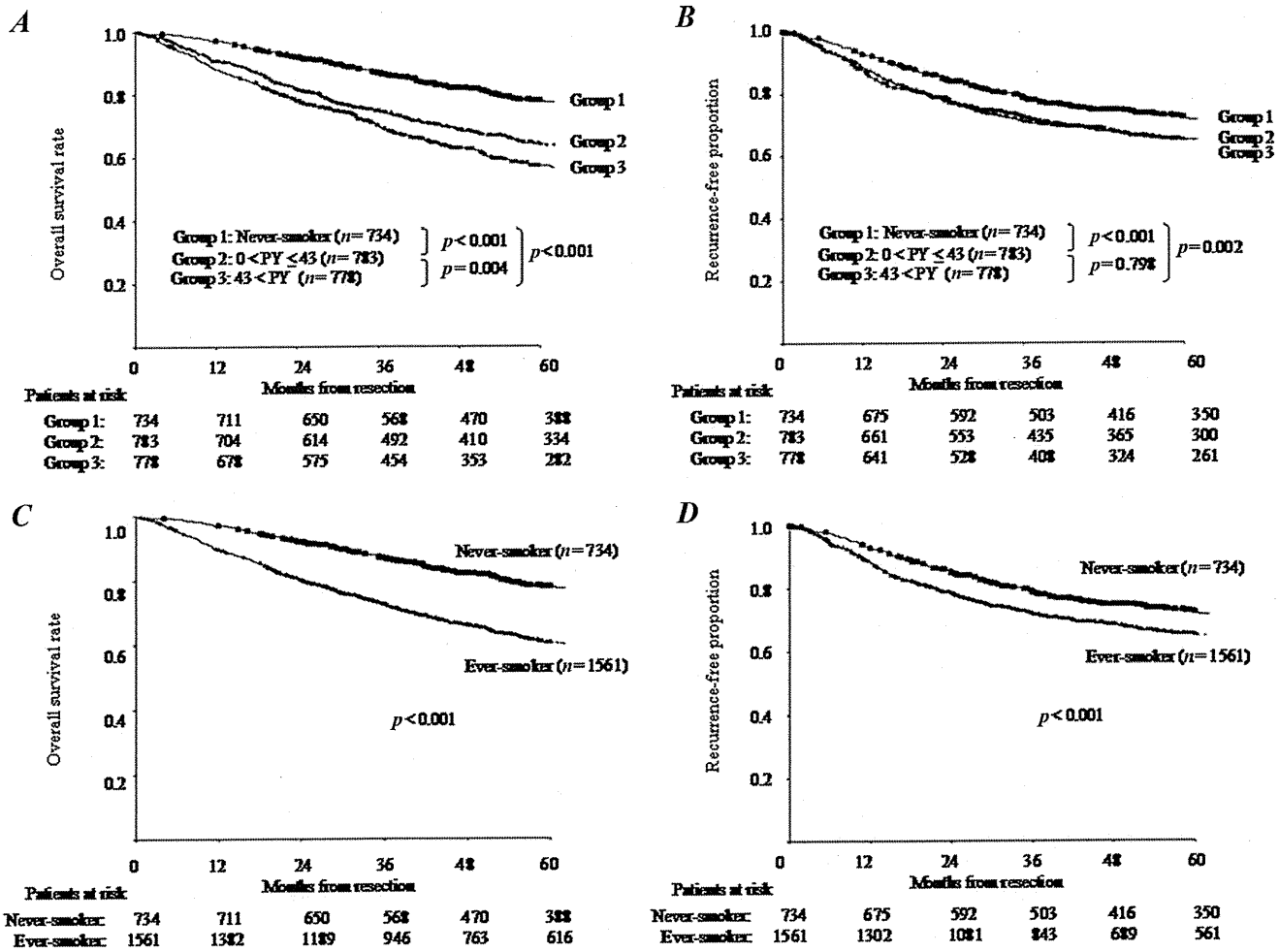

FIGURE 1. Overall survival and recurrence-free proportion (RFP) curves according to smoking status in the entire cohort. *A*, Overall survival curves according to smoking extent. *B*, RFP curves according to smoking extent. *C*, Overall survival curves according to smoking history. *D*, RFP curves according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

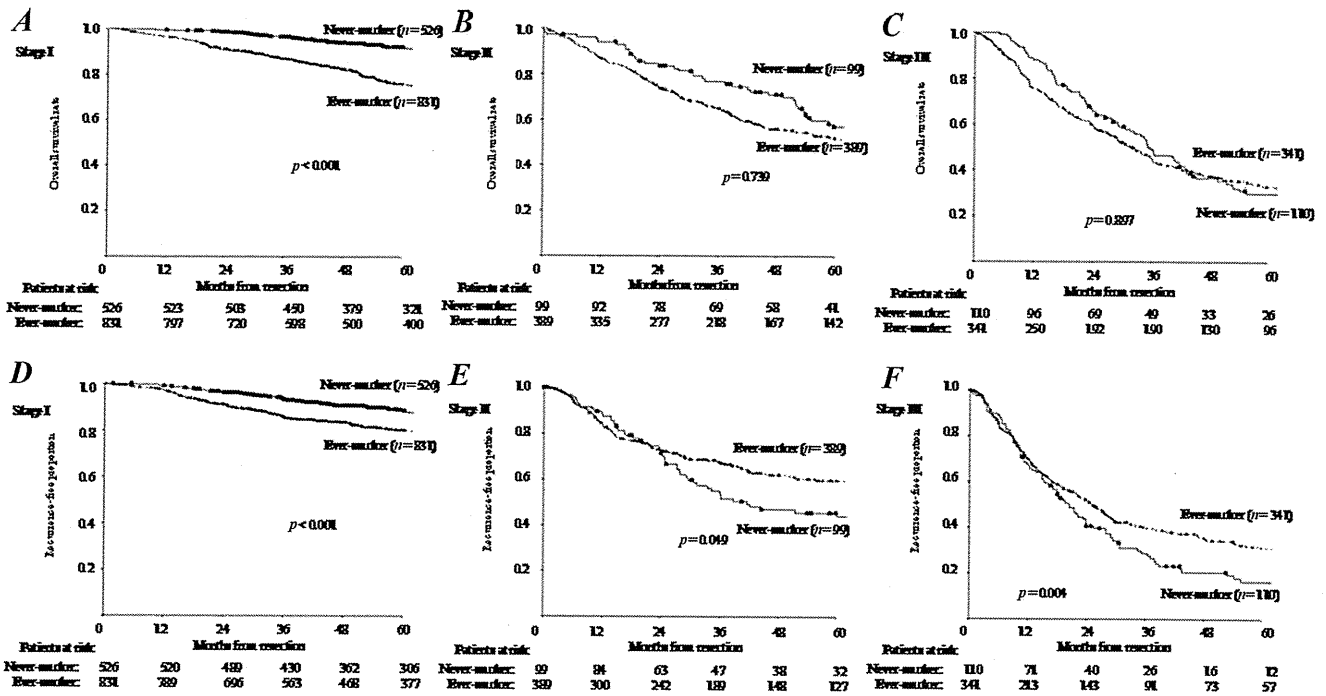

FIGURE 2. Overall survival and recurrence-free proportion (RFP) curves according to smoking history in each stage. *A*, Overall survival curves of patients with stage I non-small cell lung cancer (NSCLC). *B*, Overall survival curves of patients with stage II NSCLC. *C*, Overall survival curves of patients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

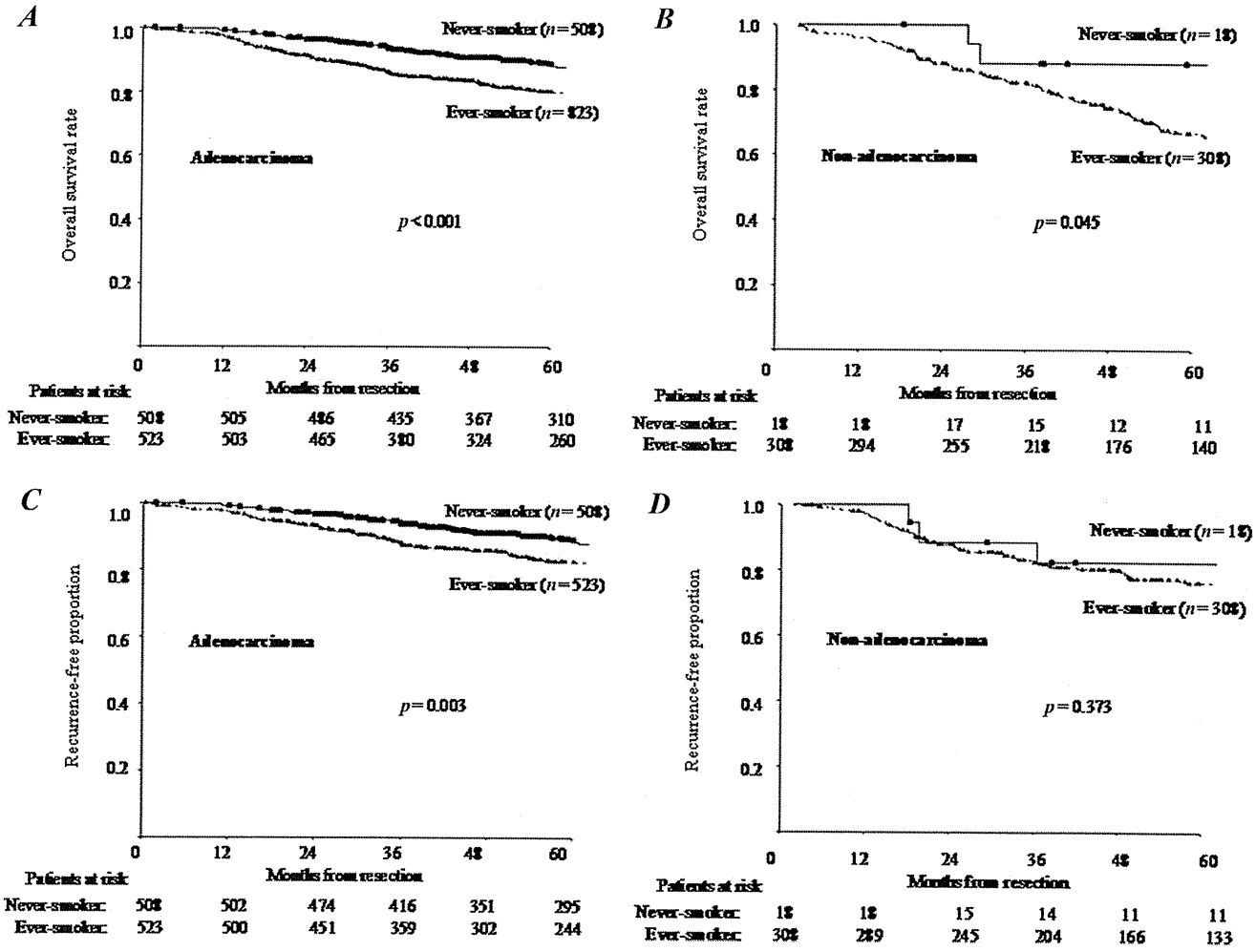

FIGURE 3. Overall survival and recurrence-free proportion (RFP) curves according to smoking history in patients with stage I non-small cell lung cancer. *A*, Overall survival curves of adenocarcinoma patients. *B*, Overall survival curves of nonadenocarcinoma patients. *C*, RFP curves of adenocarcinoma 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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Differences Between Squamous Cell Carcinoma and Adenocarcinoma of the Lung: Are Adenocarcinoma and Squamous Cell Carcinoma Prognostically Equal?

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Objective: We analyzed pulmonary squamous cell carcinoma and adenocarcinoma patient survival in our single institution database, to evaluate the relationship of histologic analysis to survival and tumor aggressiveness.
Methods: We reviewed 1856 consecutive patients with surgically resected pulmonary squamous cell carcinoma or adenocarcinoma regarding their clinicopathologic characteristics, overall survival and recurrence-free proportion.
Results: In squamous cell carcinoma patients, there were more elderly male smokers and more patients with T2–4 tumors, moderately/poorly differentiated tumors, lymph node metastasis or vascular invasion than in adenocarcinoma patients. In all patients and in pN0 patients, patients with squamous cell carcinoma showed significantly poorer overall survival than those with adenocarcinoma, but there were no statistically significant differences in the recurrence-free proportion between the two histologic types. There were statistically significantly more lung cancer-specific deaths in patients with adenocarcinoma than in patients with squamous cell carcinoma ($P = 0.001$).
Conclusions: There were no differences in the development of recurrence between squamous cell carcinoma and adenocarcinoma of the lung, but considerable differences in overall survival were observed between the two histologic types. According to the stage grouping strategy of the TNM Classification for Lung and Pleural Tumours, these two histologic types need to be staged differently. This survival difference, however, may reflect the difference in patient background rather than in biologic aggressiveness between the two histologic types.

Key words: histologic type – prognosis – squamous cell carcinoma – adenocarcinoma – TNM classification

INTRODUCTION

Squamous cell carcinoma and adenocarcinoma are the two major histologic types of non-small cell lung cancer. Patients with adenocarcinoma were known to result in poorer prognosis than those with squamous cell carcinoma (1,2). However, a recent increase in the use of computed tomography (CT) has enabled small adenocarcinoma detection on a screening basis, and many of these small adenocarcinomas are relatively dormant bronchioloalveolar carcinomas and have favorable outcome (3). This may be one reason why patients with squamous cell carcinoma are known today to have a poorer prognosis than those with adenocarcinoma following surgical resection (4).

Squamous cell carcinoma mostly develops in smokers, in whom life-threatening co-morbidities often develop, which may also explain the poorer survival rates of patients with

squamous cell carcinoma compared with those with adenocarcinoma. However, differences in biological aggressiveness between squamous cell carcinoma and adenocarcinoma of the lung are not well understood.

In esophageal cancer staging, squamous cell carcinoma and adenocarcinoma are classified differently in the 7th Edition of the Cancer Staging Manual of the American Joint Committee on Cancer (5–7). In lung cancer, however, prognostic differences in histologic types are not taken into consideration in the latest TNM classification (8).

We retrospectively analyzed the survival differences between squamous cell carcinoma and adenocarcinoma of the lung, in an attempt to identify the prognostic impact of histologic difference and to incorporate it in future staging systems, based on our patient database.

PATIENTS AND METHODS

From July 1992 through December 2006, 1856 consecutive patients with pulmonary squamous cell carcinoma or adenocarcinoma underwent complete resection at our institution. We defined complete resection as segmentectomy or greater, with systematic ipsilateral hilar and mediastinal lymph node dissection but with no evidence of residual cancer either macroscopically or histologically. Patients who had induction chemotherapy, radiotherapy or both, patients with evidence of residual tumor at the surgical margin or patients with malignant effusion or distant metastasis verified intraoperatively or by means of postoperative pathologic examination were excluded from this study.

Cases were pathologically staged based on the 7th Edition of the TNM Classification for Lung and Pleural Tumours (8). Histopathologic studies were done according to the World Health Organization criteria (9). We reviewed the medical records of all patients for the following clinicopathologic factors: age, gender, smoking history (never or ever smoker), pathological differentiation, pathological T stage, pathological N stage, vascular invasion and lymphatic permeation.

Student's *t*-test was used to evaluate the relationships between histologic type (squamous cell carcinoma or adenocarcinoma) and age. Fisher's exact test was used to evaluate the relationships between histologic type and other clinicopathologic factors. We compared overall survival and recurrence-free proportion between squamous cell carcinoma and adenocarcinoma in all patients, in pN0 patients, in pT1N0 patients, in pT2N0 patients and in pT3/4N0 patients. When we analyzed recurrence-free proportion, we excluded 249 cases from this study because their recurrence data were incomplete. The survival rates and recurrence-free proportions were calculated using the Kaplan–Meier method, and univariate analyses were performed with the log-rank test. Multivariate analyses were performed by using the Cox proportional hazards model. Zero time was the date of pulmonary resection. The endpoint of overall survival was defined as the date of death from any cause, and the last follow-up observation was censored when the patient was alive or lost to follow-up. The endpoint of recurrence-free proportion was defined as the date when recurrence was confirmed. 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 CT 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 CT have also been performed when appropriate. We diagnosed recurrence based on the findings of physical examination and diagnostic imaging and confirmed the diagnosis histologically when clinically feasible. The date of recurrence was defined as the date of cytohistological proof. However, in cases diagnosed on the basis of clinicoradiological findings, the date of recurrence was defined as the date of identification by a physician. The last follow-up observation was censored when the patient was recurrence-free or lost to follow-up. Patients who died from causes other than lung cancer recurrence were also censored on the date of death.

All P values were two-sided, and P values <0.05 were considered to represent statistically significant differences. Survival analyses were performed on SPSS software (Dr SPSS II for Windows, Standard Version 11.0, SPSS Inc., Chicago, IL, USA).

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

RESULTS

Patient Characteristics

The patient characteristics are shown in Table 1. In squamous cell carcinoma patients, compared with adenocarcinoma patients, there were more elderly male smokers and more patients with T2–4 tumors, moderately/poorly differentiated tumors, lymph node metastasis or vascular invasion. In pN0 patients ($n = 1328$), there were more elderly male smokers and more patients with T2–4 tumors, moderately/poorly differentiated tumors or vascular invasion in squamous cell carcinoma patients.

Overall Survival Differences

Patients with squamous cell carcinoma showed significantly poorer overall survival than those with adenocarcinoma in all patients and in pN0 patients (Figs 1A and 2A). The results of multivariate analyses of the statistically significant characteristics listed in Table 1 are summarized in Table 2. Age, smoking history, pathological T classification, vascular

Table 1. Patient characteristics

Patient characteristics	All patients				pN0 patients			
	AD	SQ	*P*-value	Total	AD	SQ	*P*-value	Total
Age								
Median (range)	65 (32–90)	69 (31–88)	<0.001[a]		65 (32–90)	70 (31–88)	<0.001[a]	
Sex								
Men	731 (52)	418 (90)		1149 (62)	521 (51)	263 (89)		784 (59)
Women	662 (48)	45 (10)	<0.001[b]	707 (38)	510 (49)	34 (11)	<0.001[b]	544 (41)
Smoking history								
Never smoker	617 (44)	12 (3)		629 (34)	485 (47)	9 (3)		494 (37)
Ever smoker	776 (56)	451 (97)	<0.001[b]	1227 (66)	546 (53)	288 (97)	<0.001[b]	834 (63)
Pathological T classification								
T1a, T1b	689 (49)	131 (28)		820 (44)	602 (58)	103 (35)		705 (53)
T2a, T2b, T3, T4	704 (51)	332 (72)	<0.001[b]	1036 (56)	429 (42)	194 (65)	<0.001[b]	623 (47)
Pathological N classification								
N0	1031 (74)	297 (64)		1328 (72)	—	—	—	—
N1, N2	362 (26)	166 (36)	<0.001[b]	528 (28)	—	—	—	—
Pathological differentiation								
Well	491 (36)	21 (5)		512 (28)	454 (44)	17 (6)		471 (36)
Moderately/poorly	892 (74)	440 (95)	<0.001[b]	1332 (72)	569 (56)	279 (94)	<0.001[b]	848 (64)
Vascular invasion								
Absent	818 (59)	150 (32)		968 (52)	732 (71)	128 (43)		860 (65)
Present	575 (41)	313 (68)	<0.001[b]	888 (48)	299 (29)	169 (57)	<0.001[b]	468 (35)
Lymphatic permeation								
Absent	964 (69)	320 (69)		1284 (69)	847 (82)	238 (80)		1085 (82)
Present	429 (31)	143 (31)	1.000[b]	572 (31)	184 (18)	59 (20)	0.444[b]	243 (18)
Total	1393	463		1856	1031	297		1328

AD, adenocarcinoma; SQ, squamous cell carcinoma; T/N classification according to the 7th Edition of the TNM Classification for Lung and Pleural Tumours; numbers in parentheses are percentages.
[a]Student's t-test.
[b]Fisher's exact test.

invasion and lymphatic permeation were significant prognostic factors in all patients and in pN0 patients. Pathological N classification was a significant prognostic factor in all patients. Sex, pathological differentiation and histologic type were not significant prognostic factors in any patients or in pN0 patients.

Although patients with squamous cell carcinoma showed significantly poorer overall survival than those with adenocarcinoma in pT1N0 patients and in pT2N0 patients (Fig. 3A and C), no statistically significant differences were observed in pT3/4N0 patients ($P = 0.841$; Fig. 3E).

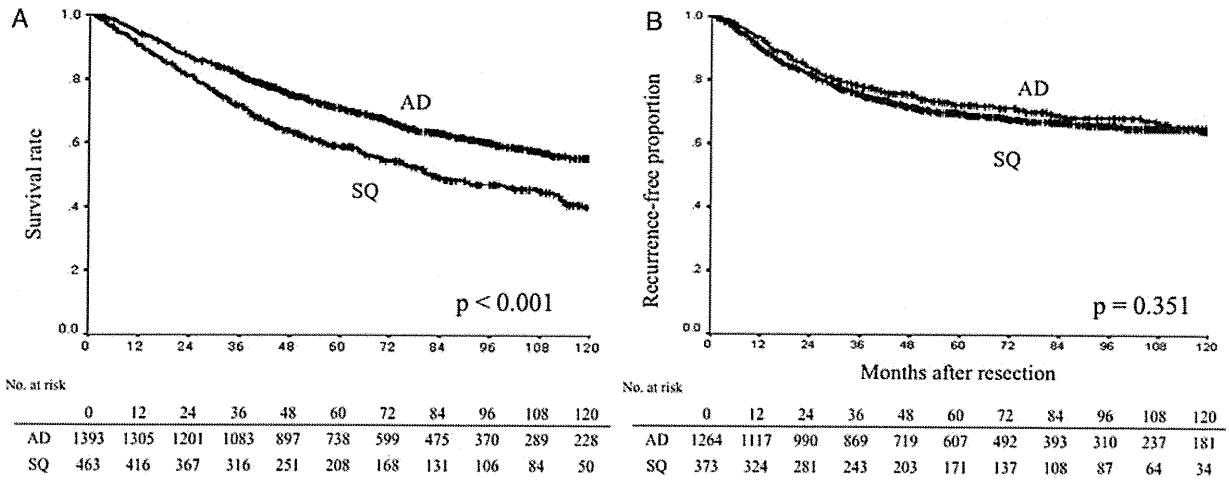
Recurrence-free Proportion Differences

There were no statistically significant differences in recurrence-free proportion between adenocarcinoma and squamous cell carcinoma in any patients ($P = 0.351$; Fig. 1B) or in pN0 patients ($P = 0.715$; Fig. 2B).

In pT1N0 patients, patients with squamous cell carcinoma showed significantly poorer recurrence-free proportion than those with adenocarcinoma (Fig. 3B). In pT2N0 patients, there was no statistically significant difference in recurrence-free proportion between the two histologic types ($P = 0.098$; Fig. 3D). In pT3/4N0 patients, patients with adenocarcinoma showed significantly poorer recurrence-free proportion than those with squamous cell carcinoma (Fig. 3F).

Causes of Death

There were 638 patients whose causes of death were identified in our cohort. There were significantly more lung cancer-specific deaths in adenocarcinoma patients than in squamous cell carcinoma patients ($P = 0.001$; Table 3).



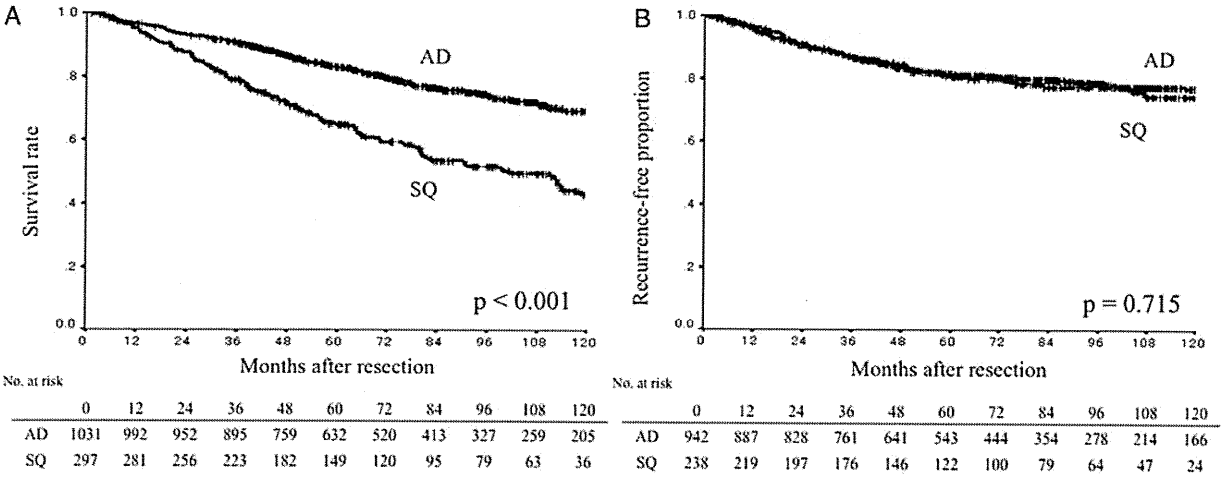
No. at risk

	0	12	24	36	48	60	72	84	96	108	120
AD	1393	1305	1201	1083	897	738	599	475	370	289	228
SQ	463	416	367	316	251	208	168	131	106	84	50

No. at risk

	0	12	24	36	48	60	72	84	96	108	120
AD	1264	1117	990	869	719	607	492	393	310	237	181
SQ	373	324	281	243	203	171	137	108	87	64	34

Figure 1. Overall survival and recurrence-free proportion between squamous cell carcinoma and adenocarcinoma in all patients. (A) Overall survival and (B) recurrence-free proportion curves of squamous cell carcinoma and adenocarcinoma in all patients. AD, adenocarcinoma; SQ, squamous cell carcinoma.



No. at risk

	0	12	24	36	48	60	72	84	96	108	120
AD	1031	992	952	895	759	632	520	413	327	259	205
SQ	297	281	256	223	182	149	120	95	79	63	36

No. at risk

	0	12	24	36	48	60	72	84	96	108	120
AD	942	887	828	761	641	543	444	354	278	214	166
SQ	238	219	197	176	146	122	100	79	64	47	24

Figure 2. Overall survival and recurrence-free proportion between squamous cell carcinoma and adenocarcinoma in pN0 patients. (A) Overall survival and (B) recurrence-free proportion curves of squamous cell carcinoma and adenocarcinoma in pN0 patients.

Table 2. Multivariate analyses of overall survival

Patient characteristics	All patients		pN0	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (>65/≤65)	1.641 (1.414–1.905)	<0.001	2.152 (1.728–2.680)	<0.001
Sex (men/women)	1.023 (0.805–1.300)	0.853	1.054 (0.768–1.446)	0.744
Smoking history (ever smoker/never smoker)	1.429 (1.104–1.848)	0.007	1.661 (1.166–2.365)	0.005
Pathological T stage (T2 + 3 + 4/T1)	1.988 (1.653–2.391)	<0.001	2.267 (1.772–2.900)	<0.001
Pathological N stage (N1 + 2/N0)	2.182 (1.844–2.582)	<0.001	—	—
Pathological differentiation (moderately + poorly/well)	1.185 (0.943–1.490)	0.145	1.180 (0.888–1.567)	0.255
Vascular invasion (present/absent)	1.572 (1.301–1.900)	<0.001	1.811 (1.426–2.301)	<0.001
Lymphatic permeation (present/absent)	1.352 (1.148–1.592)	<0.001	1.375 (1.092–1.731)	0.007
Histologic type (SQ/AD)	0.875 (0.737–1.039)	0.128	1.095 (0.866–1.385)	0.448

HR, hazard ratio for death; CI, confidence interval.

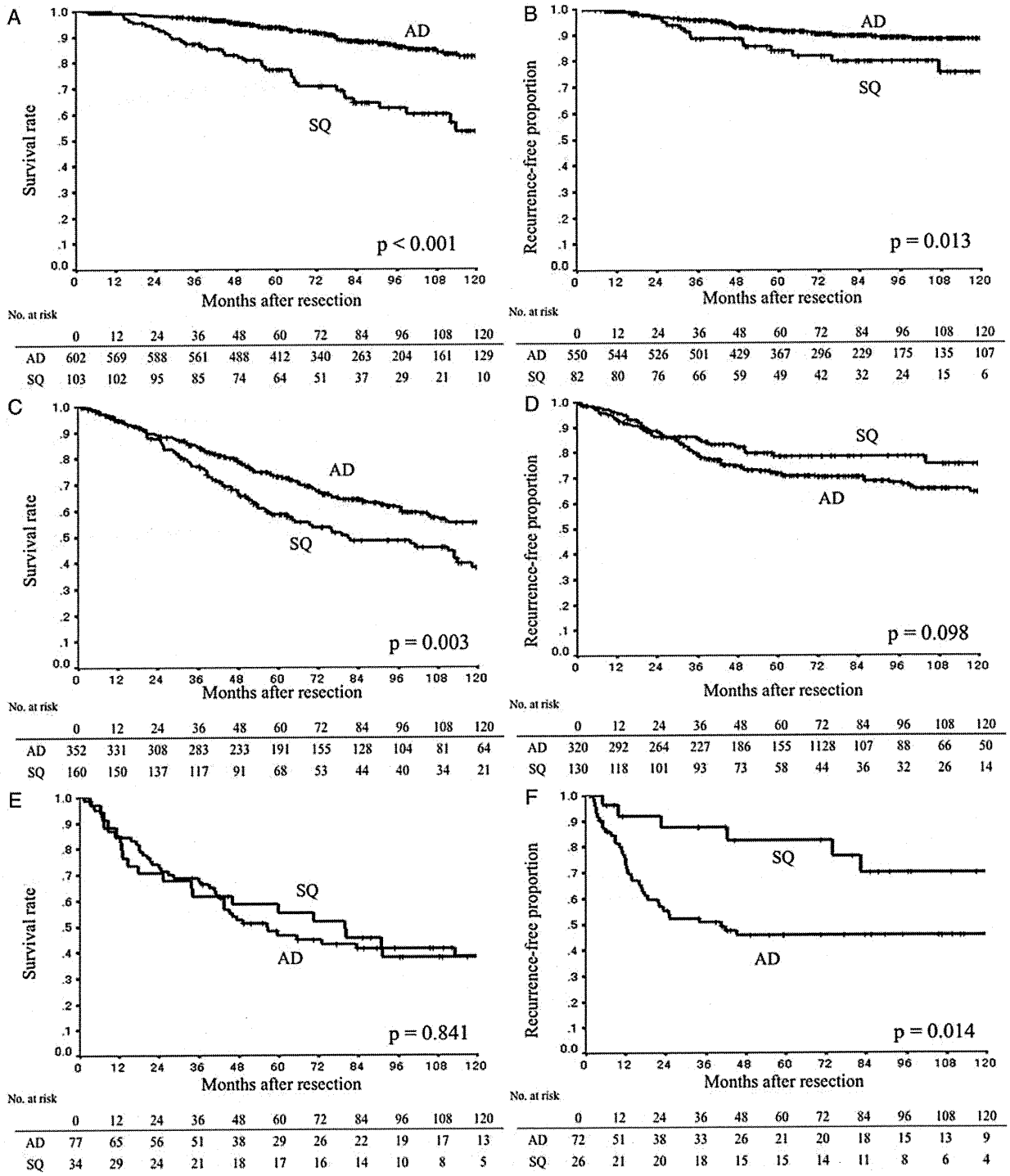


Figure 3. Overall survival and recurrence-free proportion between squamous cell carcinoma and adenocarcinoma in pT1N0 patients, in pT2N0 patients and in pT3/4N0 patients. (A) Overall survival and (B) recurrence-free proportion curves in pT1N0 patients. (C) Overall survival and (D) recurrence-free proportion curves in pT2N0 patients. (E) Overall survival and (F) recurrence-free proportion curves in pT3/4N0 patients.

DISCUSSION

We set out to determine the relationship of histologic analysis to survival and tumor aggressiveness in pulmonary squamous cell carcinoma and adenocarcinoma. Patients with pulmonary squamous cell carcinoma are known today to have a poorer prognosis than those with adenocarcinoma after surgical resection (4). Squamous cell carcinoma mostly develops in smokers

Table 3. Causes of death

Characteristics	Total	AD	SQ	*P* value
Lung cancer-specific deaths	479	355 (79)	124 (66)	
Deaths from other causes	159	96 (21)	63 (34)	0.001[a]
Total	638	451	187	

[a]Fisher's exact test; numbers in parentheses are percentages.

in whom life-threatening co-morbidities also often develop, including atherosclerotic cardiovascular events, chronic obstructive pulmonary disease and cerebral infarction (10), which may explain the poorer survival of patients with squamous cell carcinoma compared with those with adenocarcinoma. In the present study, there were significantly more patients who died of causes other than lung cancer in squamous cell carcinoma than in adenocarcinoma. However, it remains unclear whether biological aggressiveness differs between squamous cell carcinoma and adenocarcinoma of the lung.

In the present study, there were significantly more patients with squamous cell carcinoma than those with adenocarcinoma among smokers. In patients with squamous cell carcinoma, there were significantly more T2–4 patients and patients with lymph node metastases or vascular invasion. There were statistically significant differences in overall survival between adenocarcinoma and squamous cell carcinoma patients in all patients and in pN0 patient cohorts. However, when we analyzed recurrence-free proportion to exclude any possible influence of non-cancer-specific death and to compare biological aggressiveness between squamous cell carcinoma and adenocarcinoma, we found that there were no statistically significant differences in any patients or in pN0 patients. There were significantly more deaths from causes other than lung cancer in patients with squamous cell carcinoma than in those with adenocarcinoma.

These results indicate that although squamous cell carcinoma developed more frequently among smokers and was more advanced and invasive when resected compared with adenocarcinoma, its biological aggressiveness was not significantly different from adenocarcinoma. The poorer overall survival in patients with squamous cell carcinoma than those with adenocarcinoma seemed to be attributable to advanced and invasive cancer status on resection and smoking/age-related co-morbidities.

We also analyzed overall survival and recurrence-free proportion in each pathological T stage in pN0 patients to compare biological aggressiveness between squamous cell carcinoma and adenocarcinoma in each T stage. In pT1N0 patients, the patients with squamous cell carcinoma had significantly poorer survival and recurrence-free proportion than patients with adenocarcinoma. This may partly be explained by the fact that a considerable number of pT1 adenocarcinoma patients had non- or minimally invasive disease, such as bronchioloalveolar carcinoma, thereby resulting in better outcome compared with squamous cell carcinoma patients. In pT3/4 patients, on the other hand, there was no significant difference in overall survival between the two histologic types, but adenocarcinoma patients had significantly poorer recurrence-free proportion than squamous cell carcinoma patients. The poorer recurrence-free proportion of adenocarcinoma patients compared with squamous cell carcinoma patients may be interpreted that adenocarcinoma of this T status has biologically more aggressive nature than squamous cell carcinoma. However, probably because squamous cell carcinoma patients had more smoking/age-related co-morbidities and were more often killed by them than adenocarcinoma patients, there was no significant difference in overall survival.

In the 7th Edition of the TNM Classification for Lung and Pleural Tumours, stage groupings are based on overall survival (8). According to the strategy in this study, and based on our findings, squamous cell carcinoma and adenocarcinoma of the lung need to be staged differently. It is important to note, however, that the difference is likely to be due to advanced and invasive cancer status on resection and smoking/age-related co-morbidities of patients with squamous cell carcinoma, but not to biological tumor aggressiveness of squamous cell carcinoma.

There were several limitations in this study. Although the total number of consecutive patients was large (1856), the study was performed in a single institution using a homogenous Japanese ethnic group. Therefore, a multicenter trial based on various ethnic groups may be valuable. There were more well-differentiated tumors in adenocarcinomas than in squamous cell carcinomas in the present cohort. This may be another reason for the observed better prognosis in adenocarcinoma patients than in squamous cell carcinoma patients.

In conclusion, this study showed that there were no differences in the development of recurrence between squamous cell carcinoma and adenocarcinoma of the lung, but considerable differences in overall survival were observed between the two histologic types in all patients and pN0 patients. According to the stage grouping strategy of the TNM Classification for Lung and Pleural Tumours, these two histologic types need to be staged differently. This survival difference, however, may reflect the difference in patient background rather than the difference in biological aggressiveness between the two histologic types.

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Conflict of interest statement

None declared.

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Placental Growth Factor and Soluble c-Kit Receptor Dynamics Characterize the Cytokine Signature of Imatinib in Prostate Cancer and Bone Metastases

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To assess the hypothesis that the dynamics of plasma angiogenic and inflammatory cytokines after docetaxel chemotherapy with or without the c-kit/abl/platelet-derived growth factor receptor (PDGFR) inhibitor imatinib mesylate for prostate cancer are associated with outcome, the kinetics of 17 plasma cytokines before versus after chemotherapy were assessed and associations with progression-free survival (PFS) examined. After adjusting for multiple tests, significantly different declines in placental growth factor (PIGF), soluble vascular endothelial growth factor receptor-1 (VEGFR1), VEGF, and soluble c-kit were observed with docetaxel plus imatinib ($n = 41$) compared to docetaxel alone ($n = 47$). Based on a piecewise linear regression model for change in concentration of each cytokine as a function of the probability of change in p-PDGFR *in vivo*, only the dynamics of PIGF ($P < 0.0001$) and soluble c-kit ($P < 0.0001$) differed with imatinib therapy. In a Bayesian log-normal regression model for PFS, a rise in human matrix metalloproteinase 9 after docetaxel alone associated with a longer PFS. Distinct plasma angiogenic cytokines are modified by imatinib and partitioned by *in vivo* p-PDGFR dynamics after docetaxel chemotherapy for metastatic prostate cancer. Plasma PIGF and soluble c-kit kinetics are candidate biomarkers of imatinib effect. The predictive value of human matrix metalloproteinase 9 kinetics for docetaxel efficacy requires prospective validation.

Introduction

Improved outcomes with docetaxel chemotherapy for advanced castration-resistant prostate cancer are being sought with novel combinations that target putative mechanisms of disease progression and drug resistance. Preclinical modeling indicated that the platelet-derived growth factor and its receptor (PDGFR) were upregulated in prostate cancer cells proliferating within the bone microenvironment (Uehara and others 2003). The PDGFR was observed to be upregulated in endothelial cells of vasculature specifically associated with PDGF-expressing tumor, and the PDGFR inhibitor imatinib potentiated taxane efficacy via enhanced endothelial apoptosis, an antivascular effect (Uehara and others 2003; Kim and others 2006).

Contrary to preclinical estimates, a randomized controlled study that compared the efficacy of imatinib in combination with docetaxel versus docetaxel alone in men with castration-resistant prostate cancer and bone metastases showed no added benefit with imatinib (Mathew and others 2007). Unexpectedly, *in vivo* pharmacodynamic monitoring of PDGFR inhibition showed that, within the docetaxel arm, an increased probability of PDGFR activation in peripheral blood leucocytes correlated with improved progression-free survival (PFS) and overall survival (OS) (Mathew and others 2008). Rising plasma PDGF levels were associated with a decreased probability of PDGFR activation and inferior PFS (Mathew and others 2008). While the fundamental biological implications of these observations are yet to be determined, these partitioned outcomes were not equally detected in the docetaxel–imatinib combination arm.

To further explore the dynamic signature of plasma cytokines and their prognostic impact after docetaxel chemotherapy, a panel of 17 additional angiogenic and inflammatory cytokines was constructed. Individual cytokine kinetics between baseline (BL) and after docetaxel exposure, modulation by concurrent PDGF inhibitor therapy, and association with PFS outcomes were studied.

Methods

Patients

One hundred sixteen men were enrolled to a randomized study of docetaxel with placebo or imatinib for metastatic castration-resistant prostate cancer and bone metastases (Mathew and others 2007). Of these, 88 paired plasma samples

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at BL and 6 weeks later after one cycle of weekly docetaxel-based therapy at cycle 2 day 1 (C2D1) were available.

Multiplex cytokine assay

Plasma levels of all analytes described here were subsequently analyzed in duplicates using a multiplex platform, Meso Scale Discovery (MSD) (Gaithersburg, MD). The analytes were soluble c-kit receptor (c-kit), soluble vascular endothelial growth factor receptor-2 (sVEGFR2, KDR), fibroblast growth factor, VEGF, sVEGFR1, placental growth factor (PIGF), interleukin (IL)2, IL8, IL12p70, IL10, granulocyte macrophage-colony stimulating factor, interferon-γ, IL6, IL10, tumor necrosis factor-α, transforming growth factor-β, and matrix metalloproteinase-(MMP)-9. All reagents were provided with the MSD kits and tests conducted according to the manufacturer's instructions.

Statistical methods

Numerical variables were summarized using means and standard deviations, with association between pairs of variables estimated by Pearson's correlation coefficient (Snedecor and Cochran 1980). The Wilcoxon signed rank test was used for 2 sample comparisons of numerical variables (Hollander and Wolfe 1979), applying the Bonferroni P value correction for multiple tests (Snedecor and Cochran 1980). For each cytokine, the Bayesian regression model and method of Morita and others (2010) were employed to evaluate the effects of change in the cytokine level from BL to C2D1 on PFS time while accounting for the effects of hemoglobin, change in prostate-specific antigen (PSA), and change in p-PDGFR. For each patient, because p-PDGFR was measured in ~2,000 cells both at BL and at C2D1, the within-patient BL and C2D1 distributions of p-PDGFR could be estimated very reliably. Because both the BL and C2D1 distributions of p-PDGFR were clearly bimodal for all patients, the within-patient change in p-PDGFR could not be summarized usefully as the difference between the C2D1 and BL sample means. Rather, a mixture model accounting for the observed bimodality first was fit and used to estimate the differences between the right modes, denoted by δ_{Ri}, and the differences between the left modes, denoted by δ_{Li}, for the within-patient C2D1-versus-BL distributions of p-PDGFR, for each patient, $i = 1, \ldots, 88$.

In the Bayesian regression model for PFS (Morita and others 2010), δ_{Ri} was used as a covariate representing change in p-PDGFR from BL to C2D1. This was done because the values of δ_{Ri} were much larger than δ_{Li}, and moreover δ_{Ri} was strongly associated with longer PFS. Based on preliminary goodness-of-fit analyses, it was assumed that the logarithm of PFS time was normally distributed, equivalently, that PFS was lognormal. The linear component of the lognormal regression model is the mean of log(PFS time), defined as follows. For patient i and cytokine $j = 1, \ldots, 17$, denote the (BL, C2D1) cytokine values by (X_{ij}, Y_{ij}), the difference between the log-transformed cytokine values by $W_{ij} = \log(Y_{ij}) - \log(X_{ij})$, $Z_{1i} = 1$ if treatment was docetaxel+imatinib (DI) and 0 if docetaxel+placebo (DP), Z_{2i} = Hb at BL, and Z_{3i} = change in PSA from BL to C2D1. For cytokine j and patient i, the linear component was assumed to be

$$\begin{aligned}\eta_j = {} & \beta_0 + \beta_1 Z_{1i} + \{\beta_2 Z_{1i} + \beta_3(1 - Z_{1i})\} Z_{2i} \\ & + \{\beta_4 Z_{1i} + \beta_5(1 - Z_{1i})\} Z_{3i} \\ & + \{\beta_6 Z_{1i} + \beta_7(1 - Z_{1i})\} \delta_{Ri} \\ & + \{\beta_8 Z_{1i} + \beta_9(1 - Z_{1i})\} W_{ij}\end{aligned}$$

In terms of their effects on PFS time, the parameters in the linear term may be interpreted as follows:

β_1 = main DI-vs-DP treatment effect
β_2 = effect of baseline Hb in the DI arm
β_3 = effect of baseline Hb in the DP arm
β_4 = effect of change in PSA in the DI arm
β_5 = effect of change in PSA in the DP arm
β_6 = effect of change in p-PDGFR in the DI arm
β_7 = effect of change in p-PDGFR in the DP arm
β_8 = effect of change in cytokine value in the DI arm
β_9 = effect of change in cytokine value in the DP arm

Using the large (n = ~2,000 cells) within-patient p-PDGFR samples taken at BL and at C2D1, the probability of decrease in p-PDGFR after treatment, denoted by Pr(Decr), was estimated very reliably for each patient as a standardized Wilcoxon statistic. Specifically, each patient's Pr(Decr) was computed as the mean over all 0/1 indictors that each BL value of p-PDGFR was larger than each C2D1 value. For each cytokine, the following piecewise linear regression model for the BL to C2D1 change in cytokine value, W_{ij}, as a function of the estimated Pr(Decr) was fit.

$$\begin{aligned}W_{ij} &= b_{0,t} + e_{ij} \text{ if } \Pr(\text{Decr}) \le 0.5 \\ &= b_{0,t} + b_{1,t} * \{\Pr(\text{Decr}) - 0.5\} \\ &\quad + e_{ij} \text{ if } \Pr(\text{Decr}) > 0.5,\end{aligned}$$

for treatment arm t = DI or DP, where e_{ij} denotes normally distributed random measurement error. Under this model, in treatment arm t, on average the BL to C2D1 change in the cytokine value equals the constant $b_{0,t}$ if Pr(Decr) $\le$0.5 and equals the straight line $b_{0,t} + b_{1,t}$ *{Pr(Decr) – 0.5} if Pr(Decr) > 0.5. The cut-off 0.5 was used because Pr(Decr) = 0.5 corresponds to no change in the cytokine from BL to C2D1, whereas Pr(Decr) $\ge$ 0.5 and Pr(Decr) < 0.5 correspond, respectively, to the cytokine going down or up, on average. The piecewise linear form was chosen based on preliminary goodness-of-fit plots of each cytokine change as a function of Pr(Decr). Under the null hypothesis $(b_{0,DP}, b_{1,DP}) = (b_{0,DI}, b_{1,DI})$, the piecewise linear model is the same for the 2 treatment arms. This null hypothesis corresponds to the kinetics of the cytokine, as a function of Pr(Decr), not changing with the addition of imatinib to docetaxel.

Results

The distributions of the 17 plasma angiogenic and inflammatory cytokines at BL and at C2D1 within each treatment arm are summarized in Table 1. These results indicate a significant decline in IL6 and significant increases in PIGF and soluble VEGFR1 in the docetaxel-placebo arm, and a significant decline in soluble c-kit and increase in IL10 in the docetaxel-imatinib arm. Table 2 summarizes changes in cytokine values from BL to C2D1, compared between treatment arms using the Wilcoxon rank sum test. These tests indicate significantly larger declines in PIGF, soluble c-kit,