Table 2—*Clinicopathologic Characteristics and Risk Factors for Recurrence in Patients With Stage II, T2b-T3N0M0 NSCLC*

Characteristic	No. of Patients	Freedom From Recurrence Rate, 5 y, %	Univariate *P* Value	Multivariate Analysis		
				HR	95% CI	*P* value
Overall	247	61.1	...	...	...	...
Age[a], y			.489			
≤65	102	62.9				
>65	145	58.8				
Sex[a]			.733			
Female	53	59.1				
Male	194	61.7				
Smoking habits[a]			.398			
Never smoker	39	54.7				
Ever smoker	208	63.3				
CEA[a]			.897			
Within normal range	133	62.1				
Elevated	113	61.6				
Not examined	1					
Tumor size[a], cm			.367			
≤5.5	120	62.3				
>5.5	127	60.1				
Histologic type			.035[b]			
Nonadenocarcinoma	123	70.8		1	...	...
Adenocarcinoma	124	52.1		1.675	1.088-2.579	0.019[b]
Histologic differentiation[a]			.321			
Well differentiated	40	53.9				
Moderately/poorly differentiated	207	62.7				
Vessel invasion[a]			.340			
Absent	65	64.0				
Present	182	60.0				
VPI			.038[b]			
Absent	110	70.7		1	...	...
Present	137	54.8		1.689	1.086-2.626	0.020[b]
Intrapulmonary metastasis[a]			.489			
Absent	187	61.6				
Present	60	58.9				

See Table 1 legend for expansion of abbreviations.
[a]Characterstic was not included in multivariable model.
[b]Significant.

recurrence rates at 5 years after surgical resection according to clinicopathologic features in stage IIN0 and stage IIN1, respectively. On multivariate analysis using the Cox regression model, adenocarcinoma histology and presence of VPI remained statistically significant independent predictors for tumor recurrence in both groups (Tables 2, 3).

Freedom From Recurrence Rates Stratified by the Number of Risk Factors in Stage I

Subgroup analysis with a combination of three independent risk factors for tumor recurrence (histologic differentiation, presence of vessel invasion, and presence of VPI) in stage I revealed 5-year freedom from recurrence rates of 96.7%, 89.5%, 73.5%, and 62.5% for patients with zero (stage I-0), one (stage I-1), two (stage I-2), and three (stage I-3) risk factors, respectively (Fig 2). The differences in freedom from recurrence rates were statistically significant among each group (Table 4). Table 4 lists the *P* values of freedom from recurrence rate differences evaluated by log-rank test. The difference in freedom from recurrence rate was not statistically significant between patients with stage I-3 and stage II disease (Table 4).

Freedom From Recurrence Rates Stratified by the Number of Risk Factors in Stage IIN0 and Stage IIN1

Table 4 shows the results from subgroup analyses using a combination of two independent risk factors for tumor recurrence (adenocarcinoma histology and presence of VPI) in stage IIN0 and stage IIN1. The 5-year freedom from recurrence rates were 82.5%, 63.3%, and 41.1% for patients with zero (stage IIN0-0), one (stage IIN0-1), or two (stage IIN0-2) risk factors, respectively, in stage IIN0 (Fig 3). The same rates were 78.3%, 48.7%, and 40.4% for patients with zero (stage IIN1-0), one (stage IIN1-1), or two (stage IIN1-2) risk factors, respectively, in stage IIN1 (Fig 4). There

Table 3—*Clinicopathologic Characteristics and Risk Factors for Recurrence in Patients With Stage II, T1-T2bN1M0, NSCLC*

Characteristic	No. of Patients	Freedom From Recurrence Rate, 5 y, %	Univariate *P* Value	Multivariate Analysis		
				HR	95% CI	*P* value
Overall	243	53.1	...	...	...	...
Age[a], y			.688			
≤65	111	63.0				
>65	132	59.3				
Sex[a]			.606			
Female	71	60.6				
Male	172	62.4				
Smoking habits[a]			.319			
Never smoker	61	52.3				
Ever smoker	182	64.0				
CEA[a]			.632			
Within normal range	124	52.5				
Elevated	118	52.9				
Not examined	1					
Tumor size[a], cm			.655			
≤3.0	86	54.6				
>3.0	157	52.3				
Histologic type			.004[a]			
Nonadenocarcinoma	106	66.8		1	...	...
Adenocarcinoma	137	43.4		1.769	1.156-2.708	.009[a]
Histologic differentiation[a]			.874			
Well differentiated	21	44.7				
Moderately/poorly differentiated	222	54.0				
Vessel invasion[a]			.052			
Absent	30	71.7				
Present	313	51.3				
VPI			.007[a]			
Absent	142	58.5		1	...	...
Present	101	46.0		1.574	1.067-2.371	.022[a]
Number of metastatic N1 nodes			.022[a]			
Single	124	61.5		1	...	...
Multiple	119	46.2		1.38	0.936-2.034	.104
Highest level of involved lymph node station[a]			.116			
Peripheral (#12-14)	132	60.4				
Hilar (#10) or interlobar (#11)	111	47.2				

See Table 1 legend for expansion of abbreviations.
[a]Characterstic was not included in multivariable model.
[b]Significant.

was no significant difference between stage IIN0-0 and stage I, and between stage IIN1-0 and stage I (Table 4).

Discussion

Theoretically, postoperative adjuvant chemotherapy is scheduled to eliminate occult metastases and improve overall survival. Adjuvant chemotherapy is the standard treatment of different cancers, such as breast cancer[22] and colon cancer,[23] and has clearly improved overall survival. Also in NSCLC, postoperative adjuvant cisplatin-based chemotherapy now represents the standard of care for the management of patients with pathologic stage II to IIIA NSCLC diagnosed on the basis of the sixth edition TNM classification.[18,19,24] Although the seventh edition TNM classification was published in 2009, the role of adjuvant chemotherapy in stages classified according to the seventh edition has not been established. Better quantification of risk for patients with surgically resected early-stage NSCLC that are categorized based on the revised seventh edition may improve clinical decision making for adjuvant chemotherapy.

Controversy surrounds the notion of whether all patients with stage I disease should receive chemotherapy and, if not, whether there are criteria that may identify those patients who are likely to receive the most benefit from adjuvant chemotherapy.[19] In the current study, we identified three independent significant predictors for tumor recurrence by multivariate analyses: histologic differentiation, presence of vessel invasion, and presence of VPI in patients with stage I disease. Subgroup analysis with a combination of these three independent risk factors for tumor recurrence revealed 5-year freedom from

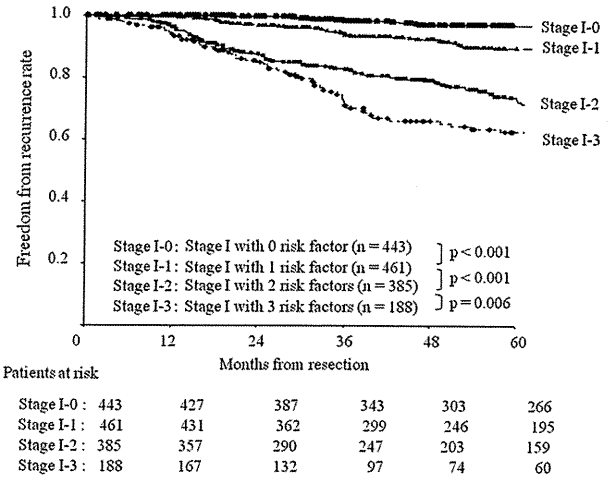

FIGURE 2. Freedom from recurrence rate curves for patients with stage I NSCLC according to the number of risk factors. See Figure 1 legend for expansion of abbreviation.

recurrence rates of 96.7%, 89.5%, 73.5%, and 62.5% for patients with zero, one, two, or three risk factors, respectively. This suggests that the histopathologic analysis, including histologic grade, vessel invasion, and VPI, may permit the identification of a subset of patients with stage I disease who have a high likelihood of tumor recurrence. The differences in freedom from recurrence rates were not statistically significant between patients with stage I disease with three risk factors and patients with stage II disease. Patients with three risk factors may be good candidates for adjuvant chemotherapy because of their higher probability for risk of tumor recurrence. In contrast, patients with stage I disease with either zero or one risk factor had a favorable outcome, and their freedom from recurrence rates were higher than that of all patients with stage I disease. Adjuvant chemotherapy may not be necessary for these patients. As the freedom from recurrence rate for patients with two risk factors is between those with either zero or one, and the three-risk factor groups, as well as between those of patients with stage I and II disease, it is unclear whether adjuvant chemotherapy is beneficial. Further study is needed to determine if there is any benefit for these patients.

Efficacy of adjuvant chemotherapy for patients with T3N0 tumor classified according to the sixth edition remains controversial.[25] Cancer and Leukemia Group B 9633[26] suggested a statistically significant survival advantage for patients with large tumors of > 4 cm. It suggests that adjuvant chemotherapy for high-risk patients without lymph node metastases may be useful for improving survival in this population. By multivariate analysis, we identified adenocarcinoma histology and presence of VPI independently as significant risk factors for tumor recurrence in patients with node-negative stage II disease. The 5-year freedom from recurrence rate of patients with node-negative stage II disease with zero risk factors was significantly higher than that of those with either one or two risk factors, and was not significantly different from that of all patients with stage I disease. Additionally, there were no significant differences in the 5-year freedom from recurrence rates between patients with node-negative stage II disease with either one or two risk factors and all patients with stage II disease. Postoperative adjuvant chemotherapy may also be unnecessary in patients with T3N0 disease without any risk factors. In contrast, patients with either one or two risk factors may be good candidates for adjuvant chemotherapy because of the higher probability of the risk of tumor recurrence.

The efficacy of adjuvant chemotherapy has been established in patients with T1-T2N1M0 NSCLC categorized according to the sixth edition TNM classification.[18,19] N1 disease represents a heterogeneous group, with 5-year survival rates roughly varying between 30% and 70%.[6-17] Some studies indicated that hilar lymph node involvement[8,12,15] and multiple lymph node metastases[8,27,28] were reported to be unfavorable prognostic factors. However, the current study did not show any statistically significant independent association between risk factors for tumor recurrence and the number of involved nodules or the location of involved lymph node stations by multivariate analysis. Instead, we identified two independently significant risk factors (adenocarcinoma histology and presence of VPI) for tumor recurrence in patients with node-positive stage II disease. The 5-year freedom from recurrence rate of patients with node-positive stage II disease without any risk factors was 78.3%, which was significantly higher than that of those with either one or two risk factors. In addition, the 5-year freedom from recurrence rates of patients with zero risk factor was not significantly different from that of all patients with stage I disease. In view of the adverse effects of chemotherapy, sparing postoperative adjuvant chemotherapy for patients with T1-2N1 tumors who lack any risk factors may be an option because of the low probability of the risk of tumor recurrence.

In the current study, several risk factors differed between patients with stage I and II disease. Especially, histology and histologic differentiation showed different impact on postoperative recurrence in patients with NSCLC between stage I and stage II. Patients with stage I disease with adenocarcinoma and well-differentiated carcinoma had higher freedom from recurrence rates. Among patients with stage II disease, however, patients with non-adenocarcinoma and moderately/poorly differentiated carcinoma had

Table 4—*Univariate Analysis of Freedom From Recurrence Rates Stratified by the Number of Risk Factors in Each Group*

Stage	N Status	No. of Risk Factors	No. of Patients	Freedom From Recurrence Rate at 5 y, %	Versus	*P* Value by Log-Rank Test
Stage I			1,477	84.2		
		0	443	96.7	Stage I	<.001[a]
					Stage II	<.001[a]
					Stage I-1	<.001[a]
					Stage I-2	<.001[a]
					Stage I-3	<.001[a]
		1	461	89.5	Stage I	.004[a]
					Stage II	<.001[a]
					Stage I-2	<.001[a]
					Stage I-3	<.001[a]
		2	385	73.5	Stage I	<.001[a]
					Stage II	<.001[a]
					Stage I-3	.006[a]
		3	188	62.5	Stage I	<.001[a]
					Stage II	.176
Stage II			490	57.7		
	N0		247	61.1		
		0	48	82.5	Stage I	.395
					Stage II	.007[a]
					Stage IIN0-1	.034[a]
					Stage IIN0-2	<.001[a]
		1	137	63.3	Stage I	<.001[a]
					Stage II	.493
					Stage IIN0-2	.054
		2	62	41.1	Stage I	<.001[a]
					Stage II	.059
	N1		243	54.1		
		0	67	78.3	Stage I	.333
					Stage II	.003[a]
					Stage IIN1-1	<.001[a]
					Stage IIN1-2	<.001[a]
		1	114	48.7	Stage I	<.001[a]
					Stage II	.045
					Stage IIN1-2	.244
		2	62	40.4	Stage I	<.001[a]
					Stage II	.010[a]

[a]Significant.

higher freedom from recurrence rates. Adenocarcinomas show a wide variety of histologic features, including bronchioloalveolar carcinoma (BAC), acinar, papillary, and solid adenocarcinoma.[21] Among these adenocarcinoma histologic subtypes, BAC is a well-differentiated subtype and reported to be associated with a favorable prognosis.[29-31] The number of never smokers with early-stage BAC subtype has recently increased in Japan. One possible explanation for why the significance of histology and histologic differentiation in postoperative outcome differs according to disease stage would be that early-stage BAC subtype adenocarcinomas are differently represented within the different substages. In addition, we focused only on recurrence as an event to offset the prognostic impact of comorbidities associated with cigarette smoking in the current study. Therefore, discrepancies in the results of this and other studies[8,12,15,27,28] may be partly attributable to this.

This study was retrospective, and there were several possible limitations in the analyses. One limitation is the relatively small number of stage II cases in the study group, as we excluded patients who had received any adjuvant or neoadjuvant chemotherapy or radiation therapy. The current study shows risk factors for tumor recurrence after resection. However, whether patients with risk factors actually benefit from adjuvant therapy is unclear from this study. Clinical trials of adjuvant chemotherapy will be needed. Another limitation is that this study is from a single Japanese institution and ethnic diversity is lacking in the 100% Japanese patient population. This study needs validation from other institutions. Despite these limitations, we believe that risk stratification using specific clinicopathologic variables for each stage by the revised TNM classification may help determine which patients will benefit most from adjuvant chemotherapy or for whom adjuvant

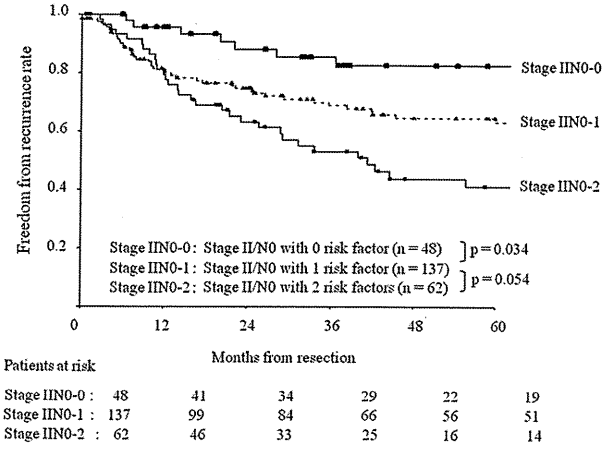

FIGURE 3. Freedom from recurrence rate curves for patients with T2b-T3N0M0, stage II NSCLC according to the number of risk factors. See Figure 1 legend for expansion of abbreviation.

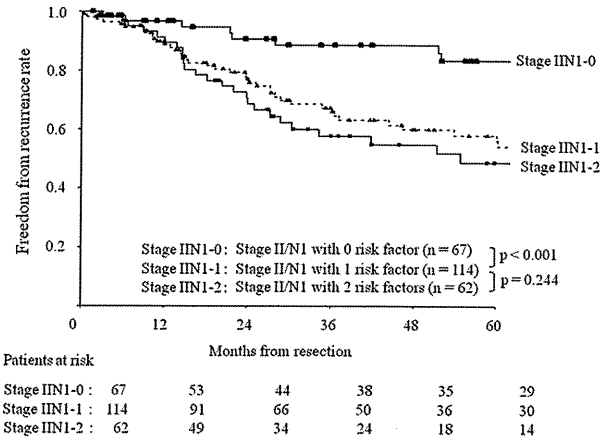

FIGURE 4. Freedom from recurrence rate curves for patients with T1-T2bN1M0, stage II NSCLC according to the number of risk factors. See Figure 1 legend for expansion of abbreviation.

chemotherapy can be spared. It will also facilitate future clinical adjuvant chemotherapy trials using new regimes in stages based on the seventh edition TNM classification.

CONCLUSION

In patients with early-stage NSCLC diagnosed on the basis of the seventh edition TNM classification, we identified risk factors for tumor recurrence that independently increase the risk of recurrence. When these factors are combined, high- or low-recurrence risk groups can be identified in each stage.

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Fibrous Stroma Is Associated with Poorer Prognosis in Lung Squamous Cell Carcinoma Patients

Yusuke Takahashi, MD,†‡ Genichiro Ishii, MD,* Tetsuhiko Taira, MD,*† Satoshi Fujii, MD,* Shinya Yanagi,* Tomoyuki Hishida, MD,† Junji Yoshida, MD,† Mitsuyo Nishimura, MD,† Hiroaki Nomori, MD,‡ Kanji Nagai, MD,† and Atsushi Ochiai, MD**

Introduction: Cancer tissue is composed of various stromal cells forming cancer-specific microenvironments. Peritumoral stroma is reportedly composed of activated fibroblasts that can influence the biological properties of tumor cells, mainly their local aggressiveness and their ability. The aim of this study was to examine whether the histological properties of peritumoral stroma are correlated with squamous cell carcinoma (SqCC) aggressiveness and clinical outcome.

Methods: A series of 220 pathological stage I lung SqCC were categorized into two types according to the histological properties of the peritumoral stroma, "fibrous stroma type" ($n = 85$), and "thin stroma type" ($n = 135$), and compared the prognostic significance. Furthermore, we compared the immunohistochemical properties of the SqCC cells surrounded by "fibrous stroma" with those of the SqCC cells surrounded by "thin stroma."

Results: The prognosis of the patients with fibrous stroma-type tumors was significantly poorer than that of the thin stroma type with regard to both recurrence-free survival ($p = 0.005$) and overall survival ($p = 0.008$). A multivariate analysis showed that the presence of a fibrous stroma was an independent prognostic factor ($p = 0.030$). Compared with the SqCC cells with a thin stroma, the SqCC cells with a fibrous stroma exhibited reduced expression of E-cadherin (55.9 versus 126.0, $p < 0.001$) and an increased expression of laminin-5γ2 (94.6 versus 25.0, $p = 0.001$), matrix metalloproteinase-7 (26.0 versus 3.50, $p = 0.009$), and c-Met (64.0 versus 36.5, $p = 0.033$).

Conclusion: SqCC with a fibrous stroma displayed higher invasive phenotype and were associated with a significantly poor prognosis. The current results suggest that the microenvironment created by both SqCC cells and the peritumoral fibroblasts may facilitate cancer aggressiveness.

*Pathology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan; †Division of Thoracic Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan; and ‡Division of General Thoracic surgery, Department of Surgery, School of Medicine, Keio University, Tokyo, Japan.

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Address for correspondence: Genichiro Ishii, MD, PhD, or Atsushi Ochiai, MD, PhD, Division of Pathology, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa City, Chiba 277-8577, Japan. E-mail: gishii@east.ncc.go.jp or aochiai@east.ncc.go.jp

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Non-small cell lung cancer (NSCLC) is the major cause of cancer-related deaths throughout the world.[1] Despite advances in biological researches and therapeutic approaches, the prognosis of patients with lung cancer remains unsatisfactory. Indeed, approximately 30% of patients with pathological stage I (p-stage I) NSCLC develop tumor recurrence and die despite having undergone a complete surgical resection.[2] The possibility that these patients may have occult metastasis at the time of surgical treatment has been suggested, highlighting the importance of evaluating the histopathological and biological factors of NSCLC that are associated with a poor prognosis.

Adenocarcinoma and squamous cell carcinoma (SqCC) are the two major histological subtypes of NSCLC; however, the pathogenesis and biological characteristics of these subtypes differ. Although many reports have described the prognostic markers for adenocarcinoma, few reports have mentioned prognostic markers for SqCC. Therefore, an analysis of the prognostic markers for lung SqCC is likely to be meaningful for the treatment of patients with these lesions.

Cancer tissue is composed of different kinds of stromal cells forming cancer-specific microenvironments. The contribution of cancer stromal fibroblasts (cancer-associated fibroblasts [CAFs]) to the development of a variety of tumors has been supported by extensive clinical evidence and the use of experimental mouse models.[3–7] In adenocarcinoma, the expressions of Podoplanin and hypoxia marker carbonic anhydrase IX by CAFs are both correlated with conventional prognostic factors and are unfavorable prognostic markers for patients with lung adenocarcinoma.[8,9] Although fibrosis is generally found around cancer nests in lung SqCC, the biological significance of CAFs is not fully understood, and the influence of CAFs on cancer progression and aggressiveness has not been thoroughly investigated.

The aim of this study was to examine whether the histological properties of peritumoral stroma are correlated with the aggressiveness of SqCC. We first analyzed the prognostic significance of the stromal fibrosis status and found that the presence of a fibrous stroma surrounding the

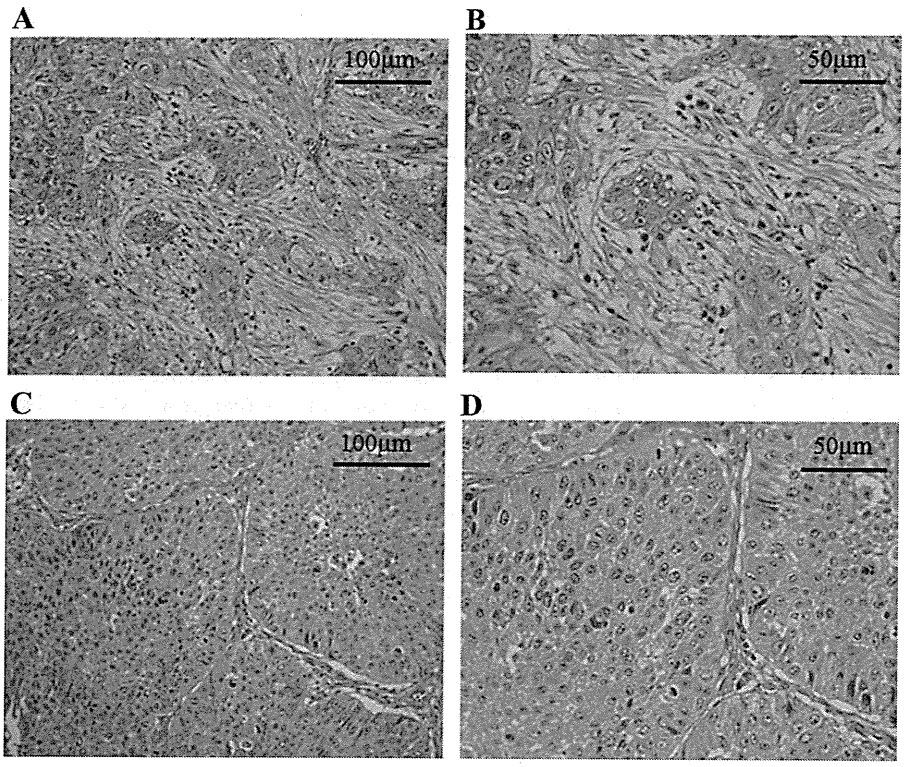

FIGURE 1. Morphological features of "fibrous stroma" and "thin stroma" (hematoxylin and eosin stained sections). *A*, "Fibrous stroma type": abundant stroma intermingled with plump fibroblast and/or collagen fibers are visible. *B*, Higher magnification of "fibrous stroma type." *C*, "Thin stroma type": the cancer cell nests were separated from each other by lymphoid stroma. *D*, Higher magnification of "thin stroma type": a narrow stroma composed of thin collagen-fiber lamellae is visible.

cancer nest was an independent indicator of a poor prognosis and was correlated with the presence of vascular invasion. Then, we examined the molecular properties of SqCC cells surrounded by fibrous stroma that might influence cancer cell biology.

Because a positive association between the presence of "fibrous stroma" and the presence of vascular invasion was observed in histological analysis, we hypothesized the fibrous stroma could facilitate invasiveness and metastatic potential of cancer cells. Therefore, we used the following antibodies that had been reported being associated with cell motility and invasive potential for immunohistochemistry: anti-E-cadherin, antilaminin-5γ2, antifibronectin, anti-matrix metalloproteinase-7 (MMP-7), and anti-c-Met.

PATIENTS AND METHODS

Patients

During the period from January 1993 to August 2005, a total of 2556 patients underwent surgical resection for primary lung cancer at the National Cancer Center Hospital East; the present study group is comprised of 220 consecutive patients with SqCC of the lung who underwent a complete resection and were diagnosed as having a p-stage I disease. Institutional Review Board-approved informed consent was obtained from all the patients. All the patients had a solitary lesion, and patients who had received preoperative chemotherapy or preoperative thoracic radiation were excluded. The preoperative evaluation included a physical examination, a blood chemistry analysis, the measurement of tumor markers, and bronchofiberscopy, chest radiography, and computed tomography examinations of the chest. The majority of patients underwent a lobectomy or pneumonectomy for the resection of the primary lesion. In cases of wedge resections or segmentectomies, a lavage sample was obtained from the surgical margin and evaluated using intraoperative rapid cytology to confirm the absence of cancer cells.

Histopathologic Analysis

The available pathology slides from all 220 surgical specimens were coded and masked for identity, then reviewed in this study by two pathologists (Y.T. and G.I.). After fixing the specimens with either 10% formalin or cold methanol and embedding them in paraffin, serial 4-μm sections were stained with hematoxylin and eosin or Victoria blue van Gieson to visualize the elastic fibers. The sections stained using Victoria blue van Gieson were examined for the presence of vascular invasion and pleural invasion. The cases were reviewed according to the current World Health Organization histological classification[10] and were staged according to the tumor node metastasis classification of the International Union Against Cancer.[11] The following conventional histopathological characteristics were evaluated: histological differentiation, pleural invasion, vascular invasion, lymphatic permeation, mitotic index, and pathological staging.

We further evaluated the following histological factors: (1) the presence of fibrous stroma surrounding the cancer nest and (2) the presence of stromal hyalinosis. Fibrous stroma surrounding the cancer cell nests was defined as stroma whose width was larger than some cancer nests and it was intermingled with plump fibroblast and/or collagen fibers (Figures 1*A*, *B*). When most cancer nests were separated from

each other by a narrow stroma, which was less than cancer nest in dimension measured in the same way, composed of thin collagen-fiber lamellae or stroma consisted of infiltrative lymphocytes, we categorized these stroma as "thin stroma" (Figures 1*C*, *D*). We defined the "fibrous stroma type" cases as those in which the predominant cancer nests were surrounded by "fibrous stroma." Meanwhile, if the stroma around the cancer nests failed to fulfill this definition, the case was regarded as a "thin stroma." Stromal hyalinosis was defined as the presence of cancer-induced stroma consisting of mainly collagen fibers, most of which were hyalinized.

Variables for Prognostic Analysis

All available clinical information was obtained from the clinical records. Central tumor location was defined as a tumor location limited to the trachea, bronchi, or segmental bronchi, and a peripheral tumor location was defined as a tumor location limited more to the periphery than to the subsegmental bronchi.[12] The following clinicopathological factors were assessed in a retrospective prognostic analysis: age (<70 years versus ≥70 years), sex, smoking index (<400 versus ≥400), forced expiratory volume in 1 second ($FEV_{1.0}$)/forced expiratory volume (<70% versus ≥70%), serum squamous cell carcinoma related antigen level (<1.5 ng/mL versus ≥1.5 ng/mL), tumor location (central versus peripheral), tumor diameter (≤30 mm versus >30 mm), histological differentiation (well to moderately versus poorly), pleural invasion (absence versus presence), vascular invasion (absence versus presence), lymphatic permeation (absence versus presence), mitotic index (≤10/10 high power field versus >10/10 high power field), cancer nest necrosis (<10% versus ≥10%), stromal hyalinosis (absence versus presence), and stroma type surrounding the cancer nest (thin stroma type versus fibrous stroma type).

Antibodies and Immunohistochemistry

After the pathologic assessment of the hematoxylin and eosin stained slides of the surgical specimens (all were pathological T1 status), the 20 most representative slides of the specimens that had been categorized as "fibrous stroma type" or "thin stroma type" were selected for the further analysis of each group. Sections (4 μm) were cut from the paraffin blocks and mounted on silanized slides. The sections were deparaffinized in xylene and dehydrated in a graded ethanol series. After washing in distilled water, the slides were placed in 0.1 M of citric acid buffer. For antigen retrieval, the slides were heated twice at 95°C for 20 minutes in a microwave oven (H2800 Microwave Processor; Energy beam Sciences, East Granby, CT) and then allowed to cool for 1 hour at room temperature. The slides were washed three times in phosphate-buffered saline (PBS) and immersed in a 0.3% hydrogen peroxidase solution in methanol for 15 minutes to inhibit endogenous peroxidase activity. After washing the slides three times in PBS, nonspecific binding was blocked by preincubation with 2% normal swine serum in PBS (blocking buffer) for 30 minutes at room temperature. Individual slides were then incubated overnight at 4°C with the following antibodies: anti-E-cadherin (diluted 1:500, clone 36; BD Bioscience, San Jose, CA), antilaminin-5γ2 (1:200, clone D4B5; Chemicon, Temecula, CA), antifibronectin (1:200, clone 568; Novocastra, Newcasyle upon Tyne, UK), anti-MMP7 (1:100, clone 141-7B2; Daiichi Fine Chemical, Toyama, Japan), and antic-Met (1:100; clone 8F11, Novocastra). The slides were again washed three times in PBS and then incubated with EnVision (Dako, Glosstrup, Denmark) for 1 hour at room temperature; after extensive washing in PBS, they were visualized in 2% 3,3′-diaminobenzidine in 50 mM of Tris buffer (pH 7.6) containing 0.3% hydrogen peroxidase. Finally, the slides were counterstained with Meyer's hematoxylin, dehydrated, and mounted. We used positive control of each antibody in the immunohistochemical study as follows: E-cadherin: bronchial epithelium, laminin-5γ2: basement membrane of bronchial epithelium, fibronectin: connective tissue of alveolar septa, MMP7: human lung adenocarcinoma, and c-Met: bronchial epithelium.

Immunohistochemical Scoring

All the tissue sections that were stained were semiquantitatively scored and evaluated independently under a light microscope, as previously described[13]; two pathologists (Y.T. and G.I.) who had no clinicopathological information regarding the cases performed the evaluations. The labeling score was calculated by multiplying the percentage of positive tumor cells in four high-power fields (×200) from the most representative areas of each slide (0–100%) by the staining intensity level (0, negative; 1, weak; and 2, strong). We confirmed that positive control tissues were stained by each antibody. When the evaluation results differed between the two observers, the specimens were examined using a multiheaded-microscope, and a consensus was obtained.

Statistical Analysis

Overall survival (OS) was measured from the date of surgery until the date of death from any cause or the date on which the patient was last known to be alive. Survival curves were plotted according to the Kaplan-Meier method and compared using the log-rank test in a univariate analysis. The recurrence-free survival (RFS) time was measured as the interval between the date of surgery and the date of recurrence, or the date of death from any cause or the most recent date on which the patient was last known to be alive. To determine the independent prognostic factors, a multivariate analysis was conducted using the Cox proportional hazard model. Two category comparisons were performed using the Pearson χ^2 test and the Mann-Whitney U test for quantitative data. All the tests were two sided, and p values less than 0.05 were considered statistically significant. The statistical analysis was performed using SPSS software (version 11.0; SPSS Inc., Chicago, IL).

RESULTS

Clinicopathological Characteristics of SqCC Cases with "Fibrous Stroma"

We examined the clinicopathological characteristics of the cases with fibrous stroma-type tumors (Table 1). A peripheral tumor location ($p = 0.043$), the presence of vascular invasion ($p = 0.004$), and the presence of stromal

TABLE 1. Relationship between Stroma Type and Clinicopathologic Factors

Factors	Stroma Type		p
	Thin Stroma Type	Fibrous Stroma Type	
Age (yr)			
<70	65	38	0.618
≥70	70	47	
Gender			
Male	118	75	0.855
Female	17	10	
Smoking index			
<400	12	10	0.489
≥400	123	75	
Tumor location			
Central	35	12	0.043
Peripheral	100	73	
Tumor diameter (mm)			
≤30	76	49	0.844
>30	59	36	
$FEV_{1.0}$/FEV(%)			
<70	54	30	0.489
≥70	81	55	
Histologic differentiation			
Well to moderate	99	65	0.185
Poor	46	20	
Pleural invasion			
Absence	111	64	0.214
Presence	24	21	
Vascular invasion			
Absence	79	33	0.004
Presence	56	52	
Lymphatic permeation			
Absence	113	64	0.126
Presence	22	21	
Mitotic index			
≤10/10 HPF	93	57	0.777
>10/10 HPF	42	28	

Two-category comparison was performed by Pearson's χ^2.
$FEV_{1.0}$, forced expiratory volune in 1 second; FEV, forced expiratory volume; HPF, high power field.

TABLE 2. Univariate Prognostic Analysis of Clinicopathologic Factors

Factors	N (%)	5-yr OS (%)	p
Age (yr)			
<70	103 (46.8)	75.6	<0.001
≥70	117 (53.2)	57.1	
Gender			
Male	193 (87.7)	66.2	0.780
Female	27 (12.3)	63.0	
Smoking index			
<400	22 (10.0)	66.7	0.806
≥400	198 (90.0)	64.0	
$FEV_{1.0}$/FEV(%)			
<70	84 (38.2)	65.2	0.370
≥70	136 (61.8)	67.4	
Serum SCC (ng/ml)			
<1.5	175 (79.5)	65.7	0.898
≥1.5	45 (20.5)	66.4	
Tumor location			
Central	47 (21.3)	72.0	0.095
Peripheral	173 (78.6)	64.2	
Histologic differentiation			
Well to moderate	154 (70.0)	66.0	0.601
Poor	66 (30.0)	65.9	
Pleural invasion			
Absence	175 (79.5)	69.4	0.045
Presence	45 (20.5)	51.8	
Vascular invasion			
Absence	112 (50.9)	72.7	0.140
Presence	108 (49.1)	58.7	
Lymphatic permeation			
Absence	177 (80.5)	67.1	0.669
Presence	43 (19.5)	60.5	
Mitotic index			
≤10/10 HPF	70 (31.8)	67.2	0.946
>10/10 HPF	150 (68.2)	63.2	
Cancer nest necrosis (%)			
<10	147 (66.8)	69.1	0.295
≥10	73 (33.2)	59.1	
Stromal hyalinosis			
Absence	177 (61.4)	67.2	0.380
Presence	43 (19.5)	60.2	
Stroma type			
Thin stroma type	135 (61.4)	72.3	0.008
Fibrous stroma type	85 (38.6)	55.5	

Log-rank test was used in univariate analysis.
OS, overall survival; $FEV_{1.0}$, forced expiratory volume in 1 second; FEV, forced expiratory volume; SCC, squamous cell carcinoma; HPF, high power field.

hyalinosis ($p = 0.002$) were significantly more common among the cases with fibrous stroma-type lesions.

Clinicopathological Characteristics and Univariate Analysis of p-Stage I SqCC

The study cohort included 87 men and 76 women, with a mean age of 69 years (range: 43–88 years; standard deviation: 7.9 years). Nine (4.1%) of the 220 patients underwent a wedge resection, 9 (4.1%) underwent a segmentectomy, 198 (90%) underwent a lobectomy, and 4 (1.8%) underwent a pneumonectomy (data not shown). The follow-up periods ranged from 4 to 150 months (the median follow-up for surviving patients was 61 months).

The patients' clinicopathological characteristics and the results of a univariate analysis are shown in Table 2. A male predominance and a high smoking status were notable characteristics. The clinically significant survival predictors, as shown using a univariate analysis, were age (<70 years versus ≧70 years, $p < 0.001$) and tumor diameter (≤30 mm versus >30 mm, $p = 0.031$). Pathologically, 147 patients (66.8%) had cancer nest necrosis accounting for less than 10% of the tumor, and 43 (19.5%) had stromal hyalinosis. When the cases were divided into two groups according to the

stroma type surrounding the cancer nest, the number of cases with fibrous stroma was 85 (38.6%). A univariate prognostic analysis of the pathological factors revealed that pleural invasion (absence versus presence, $p = 0.045$) and fibrous stroma type ($p = 0.008$) had prognostic significance. In contrast, the other histological factors showed no prognostic significance.

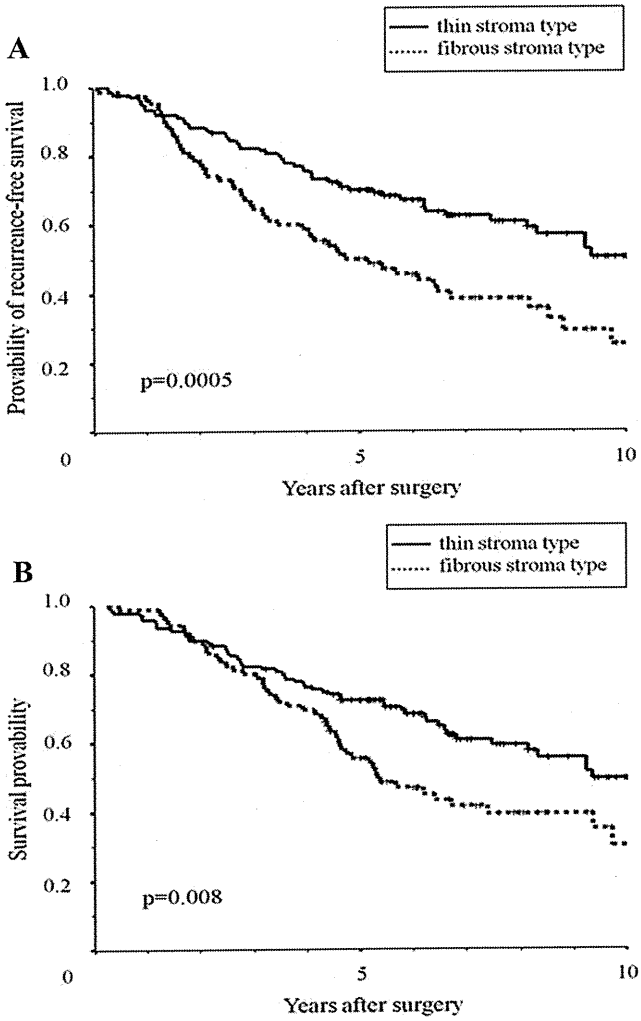

FIGURE 2. *A*, Kaplan-Meier recurrence-free survival curve for patients with pathological stage I (p-stage I) lung squamous cell carcinoma (SqCC) according to stroma type surrounding the cancer nests. *B*, Kaplan-Meier overall survival curve for patients with p-stage I lung SqCC according to the stroma type surrounding the cancer nests. Log-rank test was used in both survival analysis.

Survival Analysis According to Stroma Type

Figure 2*A* shows the RFS curves according to the results of the examination for stroma type surrounding the cancer nests (thin stroma type versus fibrous stroma type). The 5-year RFS rates for the thin stroma type and the fibrous stroma type were 70.0% and 50.0%, respectively. The RFS time for the fibrous stroma type was significantly shorter than that of the thin stroma type ($p = 0.0005$). Figure 2*B* shows the OS curves according to stroma type surrounding the cancer nests. The 5-year OS rates of the cases with the thin stroma type and the cases with the fibrous stroma were 72.3% and 55.5%, respectively. The OS of the patients with the fibrous stroma type was significantly shorter than that of the patients with the thin stroma type ($p = 0.008$).

Multivariate Analysis

To determine whether the presence of fibrous stroma surrounding the cancer nests was an independently significant prognostic factor, a multivariate analysis was performed. The four clinicopathological factors that were shown to be important survival predictors, as above, and the tumor location were included as covariates in the multivariate analysis. As shown in Table 3, the results showed that the age (<70 years versus ≧70 years, $p < 0.001$) and the stroma type surrounding the cancer nests (thin stroma type versus fibrous stroma type, $p = 0.030$) were independent prognostic factors.

Molecular Properties of Cancer Cells Surrounded by Fibrous Stroma

We performed immunohistochemical staining for the following molecular markers: E-cadherin, laminin-5γ2, fibronectin, MMP7, and c-Met. The staining results are shown in Table 4 and Figure 3. Immunoreactivity for E-cadherin was found in the cytomembrane, and the mean staining scores for the thin stroma type and the fibrous stroma type were 126.0 and 55.9, respectively (Figures 3*A*, *B*). The mean staining scores for laminin-5γ2, which was seen as positive in the cytoplasm of cancer cells, in the cancer nests surrounded by the thin stroma type and the fibrous stroma type were 25.0 and 94.6, respectively (Figures 3*C*, *D*).

Immunoreactivity for fibronectin was found in the cytoplasm of the cancer cells, and the mean staining scores in

TABLE 3. Multivariate Prognostic Analysis

Variables	Favorable	Unfavorable	Hazard Ratio	95% CI	*p*
Age (yr)	<70	≧70	2.470	1.613–3.781	<0.001
Tumor location	Peripheral	Central	1.142	0.665–1.959	0.631
Tumor diameter (mm)	≦30	>30	1.368	0.915–2.045	0.126
Pleural invasion	Absence	Presence	1.182	0.701–1.992	0.531
Stroma type	Thin stroma type	Fibrous stroma type	1.541	1.042–2.277	0.030

Multivariate analysis was conducted using the Cox proportional hazard model.
CI, confidence interval.

TABLE 4. Immunohistochemical Staining Score According to Stroma Type Surrounding Cancer Nest

Antibodies	Straining Score (Mean ± SE) Stroma Type: Thin Stroma Type	Fibrous Stroma Type	*p*
E-cadherin	126.0 ± 2.81	55.9 ± 1.44	<0.001
Laminin-5γ2	25.0 ± 1.16	94.6 ± 3.52	0.001
Fibronectin	8.41 ± 1.14	26.1 ± 1.79	0.054
MMP7	3.50 ± 0.42	26.0 ± 1.00	0.009
C-Met	36.5 ± 1.18	64.0 ± 1.76	0.033

Mann-Whitney *U* test was used in comparison between the two groups.

the thin stroma type and the fibrous stroma type were 8.41 and 26.1, respectively. MMP7 was also found in the cytoplasm of cancer cells, and the mean staining scores for the thin stroma type and the fibrous stroma type were 3.50 and 26.0, respectively (Figures 3*E*, *F*). The expression of c-Met could be clearly demonstrated in the cytoplasm of cancer cells, and the mean staining scores for the thin stroma type and the fibrous stroma type were 36.5 and 64.0, respectively (Figures 3*G*, *H*).

The scoring for E-cadherin was significantly lower for the fibrous stroma type than for the thin stroma type ($p < 0.001$). On the other hand, the laminin-5γ2 ($p = 0.001$), MMP7 ($p = 0.009$), and c-Met ($p = 0.033$) expression levels were significantly higher in the fibrous stroma type than in the thin stroma type.

DISCUSSION

Although SqCC of the lung is a major histological subtype of NSCLC, along with adenocarcinoma, the pathogenesis and biological properties of lung SqCC are not well understood. Whether any differences in the mechanism of disease progression exist according to histological subtype has also been unclear. Recent studies have suggested that tumorigenesis and progression could be influenced by tumor-associated stromal cells, including fibroblasts, leukocytes, and endothelial cells, all of which contribute to variety in the tumor microenvironment.[14–16] Several proteins expressed by CAFs may contribute to tumor aggressiveness, and may serve as unfavorable prognostic markers in lung adenocarcinoma.[8,9] In addition, several reports have suggested that the molecular properties of CAFs influence the biological features of tumor cells and the outcomes of patients.[17,18]

In the current study, patients with pathological stage I SqCC were selected because the early stage disease without lymph node metastasis would reflect the cancer cell biology more reliably. We found that the presence of fibrous stroma surrounding the cancer nests was a significant indicator of a poor prognosis and was correlated with the presence of vascular invasion. Therefore, we hypothesized the fibrous stroma could facilitate invasiveness and metastatic potential of cancer cells and investigated the expression of markers which had been reported being associated with cell motility and invasive potential. Furthermore, these SqCC cells with fibrous stroma exhibited a reduction in the expression of E-cadherin and increases in the expressions of laminin-5γ2, c-Met, and MMP7, compared with the SqCC cells with thin stroma.

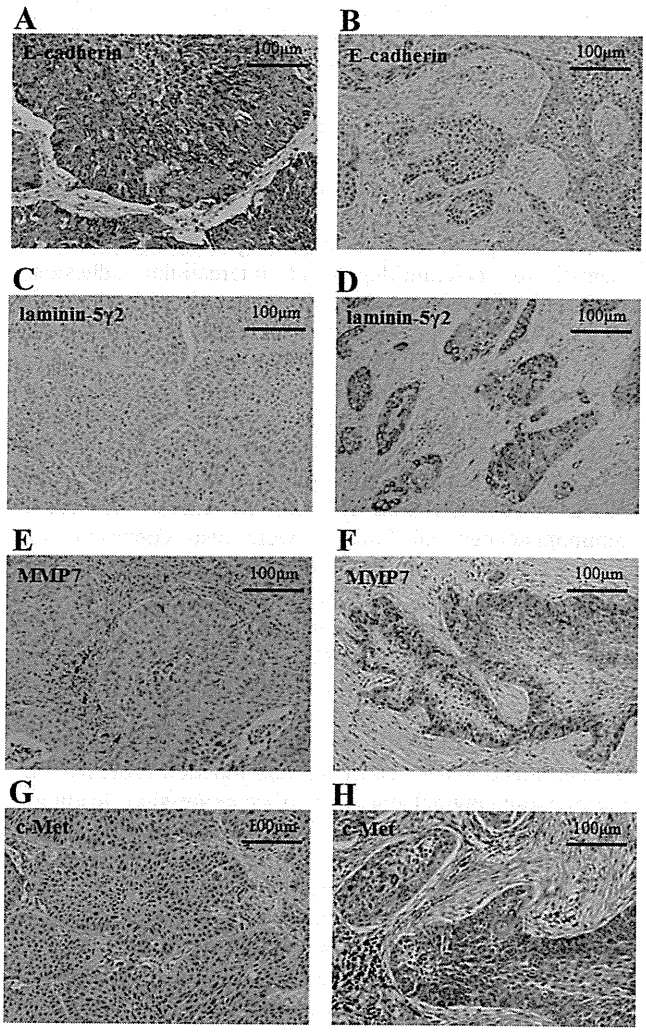

FIGURE 3. Representative immunohistochemical findings for "thin stroma type" and "fibrous stroma type." *A*, E-cadherin expression in "thin stroma type." Positive cells were found in the cancer cell nests. *B*, E-cadherin expression in "fibrous stroma type." Only a few cancer cells showed positive reactions. *C*, Laminin-5γ2 expression in "thin stroma type." Positive cells were not found. *D*, Laminin-5γ2 expression in "fibrous stroma type." A positive reaction was found in the cytoplasm of the cancer cells. Note a few budding cells around the cancer nest are also positive for laminin-5γ2. *E*, Matrix metalloproteinase-7 (MMP-7) expression in "thin stroma type." Positive cells were not found. *F*, MMP-7 expression in "fibrous stroma type." Positive cells were found, especially in the periphery of the cancer cell nests. *G*, c-Met expression in "thin stroma type." A few cancer cells showed positive immunoreactions for c-Met. *H*, c-Met expression in "fibrous stroma type." Positive cells were scattered, especially at the periphery of the cancer cell nests.

In general, tumor progression consists of multisteps, which contain loss of epithelial junctions, increase of cell motility, and invasiveness to surrounding organs and vessels. And these steps should be caused by molecular changes of cancer cells.[19] Because a positive association between the presence of "fibrous stroma" and the presence of vascular invasion was observed, we examined expression of molecular markers as above.

E-cadherin, a transmembrane glycoprotein, which is essential for calcium-dependent intercellular adhesion.[20] Fragments of laminin-5γ2 chain is known to regulate cell survive and migration.[21] MMP7 has reported to be responsible for degradation of extra cellular matrix component and play important role in tumor progression.[22] The cancer cells that were surrounded by fibrous stroma clearly exhibited a reduced expression of E-cadherin and increased expressions of laminin-5γ2 and MMP7, corresponding to the molecular features of increased motility and invasiveness.[23] The current immunohistochemical findings were also consistent with those of a previous report that described a higher expression of the similar markers, associated with a higher rate of vessel invasion and a poor prognosis in patients with NSCLC.[24] Our results suggested that cancer cells surrounded by fibrous stroma was possibly associated with a part of epithelial mesenchymal transition, which is defined as a transformation of epithelial cells into a mesenchymal cell-like morphology with combined loss of epithelial cell junction proteins and a gain of mesenchymal lineage marker expression, leading to cells with increased motility.

Furthermore, we found the increased expression of c-Met in SqCC cells with fibrous stroma. A transmembrane tyrosine kinase, c-Met was identified as the receptor for hepatocyte growth factor (HGF). HGF/c-Met signaling modulates cell spread and invasiveness, and the over expression of c-Met facilitates the disruption of E-cadherin junctions.[25] A recent study reported that an increase in HGF secretion from CAFs activates c-Met in esophageal SqCC cells and that this pathway promotes cell invasion to the matrix in three-dimensional organotypic cultures. Moreover, the constitutive activation of Met resulted in a phenotypic change in the cells, with decreased cell-cell contact and increased mesenchymal features such as an elongated cellular morphology.[26] Our results could be regarded as supporting these in vitro findings.

Tumor budding is a histological feature that is defined as a single cell or clusters of up to four or five cancer cells at the invasive tumor front.[27] The process of tumor budding is linked to the motile phenotype, which involves a reduction in intercellular contacts and cell-matrix contacts and seems to be the first event in tumor invasion and metastasis.[28,29] Yamaguchi et al.[30] demonstrated that budding cells in lung adenocarcinoma displayed decrease expression of E-cadherin and increase expression of laminin-5γ2. In the current study, cancer cells forming solid nests with fibrous stroma also presented similar expression of markers, which indicated the aggressiveness of tumor cells, even though they did not have the morphological features of mesenchymal cells or tumor budding (data not shown).

In conclusion, we clearly showed that SqCC with a fibrous stroma type was associated with a poor prognosis among patients with p-stage I lung SqCC after complete resection. Elucidating whether the peritumoral stroma of lung SqCC contributes to cancer progression would be meaningful for the development of treatment targeting the tumor microenvironment and tailored to the histological subtype. Furthermore, our findings also suggested that the microenvironment created by SqCC cells and peritumoral fibrous stroma is closely correlated with cancer aggressiveness, and this is the first report to indicate that the nature of the stroma surrounding the cancer nests contributes to the tumor invasion and a poor prognosis among lung SqCC cases. A more mechanistically oriented experimental approach would be required for elucidation of the mechanism and the key regulator(s) of the interaction between SqCC cells and CAFs which lead to useful therapeutic options in years to come.

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Original Research

LUNG CANCER

Prognostic Impact of Histology on Early-Stage Non-small Cell Lung Cancer

Ryo Maeda, MD; Junji Yoshida, MD; Genichiro Ishii, MD; Tomoyuki Hishida, MD; Mitsuyo Nishimura, MD; and Kanji Nagai, MD

Background: **The purpose of this study is to evaluate the significance of histology as a predictor of recurrence after resection in patients with early-stage non-small cell lung cancer.**
Methods: **A total of 1,870 consecutive patients in stage I and II with adenocarcinoma or squamous cell carcinoma (SCC) who underwent complete tumor resection with systematic lymph node dissection between August 1992 and December 2007 were included.**
Results: **In patients with SCC, significantly more tumors were stage IB or higher. Ever smokers were more common in patients with SCC, and more patients with SCC died of other diseases. In stage IA, a statistically significant difference in the 5-year recurrence-free probability was observed between adenocarcinoma and SCC (91.4% and 82.6%, respectively; $P < .001$), whereas no such difference was observed in stage IB (74.4% and 73.6%, respectively; $P = .934$). In stage II, the 5-year recurrence-free probability for adenocarcinoma was significantly lower than that for SCC (47% and 73%, respectively; $P < .001$). In stage IA, patients with predominantly bronchioloalveolar carcinoma subtype were more common compared with stage IB or higher in patients with adenocarcinoma.**
Conclusions: **It is important to offset the prognostic impact of comorbidities associated with cigarette smoking because more patients with SCC died of other diseases. When evaluating its significance as a predictor of recurrence stratified by stage, histology showed a different impact on postoperative recurrence within different substages. Histologic subtype distribution was different among substages in patients with adenocarcinoma. Disease stages should be considered while evaluating histology as a predictor of recurrence.** ***CHEST 2011; 140(1):135–145***

Abbreviations: BAC = bronchioloalveolar carcinoma; IVI = intratumoral vascular invasion; NSCLC = non-small cell lung cancer; SCC = squamous cell carcinoma; VPI = visceral pleura invasion

The major histologic types of lung cancer are small cell carcinoma, adenocarcinoma, squamous cell carcinoma (SCC), and large cell carcinoma. Among these histologic types, adenocarcinoma and SCC are the most frequent and account for > 80% of resected lung cancers.[1] Most studies comparing adenocarcinoma to SCC have not shown histology as an independent prognostic factor, whereas some studies have indicated that SCC shows a significantly unfavorable prognosis compared with adenocarcinoma in early-stage non-small cell lung cancer (NSCLC).[2,3] Although substantial clinical and basic science research reported the prognostic factors for patients with lung cancer, most such factors are not readily observed in routine clinical practice.[4] On the other hand, histology is an easily available prognostic factor.

Cigarette smoking is well known to be a main cause of lung cancer and is associated with all histologic types of lung cancer.[5-7] Most studies suggest that

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Affiliations: From the Department of Thoracic Oncology (Drs Maeda, Yoshida, Hishida, Nishimura, and Nagai), and Department of Pathology, Research Center for Innovative Oncology (Dr Ishii), National Cancer Center Hospital East, Kashiwa, Chiba, Japan.
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Correspondence to: Kanji Nagai, MD, Department of Thoracic Oncology, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa, Chiba, 277-8577, Japan; e-mail: knagai@east.ncc.go.jp

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cigarette smoking is more strongly associated with SCC than with adenocarcinoma.[5] One of the possible reasons that SCC has a poorer prognosis compared with adenocarcinoma[2,3] is that smoking-associated factors, such as low socioeconomic status,[8] poor nutirition,[9] comorbidity,[10] and impaired immune function,[11] may be contributory to the poorer survival in SCC compared with adenocarcinoma. It remains unclear whether histology is important in predicting recurrence after curative resection.

The purpose of this study is to evaluate the prognostic impact of histology in patients with early-stage NSCLC undergoing complete tumor resection. If we use all-cause mortality as the outcome measure, it is possible that smoking-related diseases confound the relationship between histology and mortality. Therefore, to offset the prognostic impact of comorbidities associated with cigarette smoking and to focus on evaluating its significance as a predictor of recurrence after tumor resection, we investigated recurrence-free probability in addition to overall survival rate.

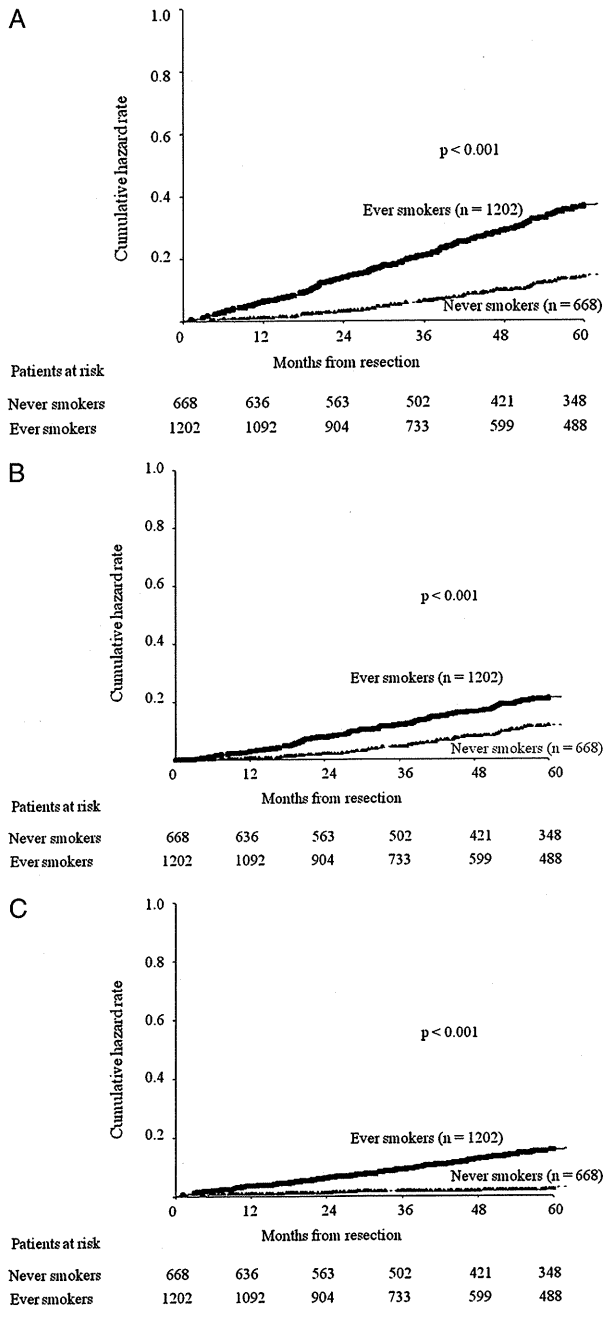

FIGURE 1. A, Cumulative hazard rate curves of all-cause deaths according to smoking history. B, Cumulative hazard rate curves of death from lung cancer according to smoking history. C, Cumulative hazard rate curves of death from other causes according to smoking history.

MATERIALS AND METHODS

Patients

A total of 1,904 consecutive patients in stage I and II with adenocarcinoma or SCC underwent complete tumor resection with lobectomy or a more extensive surgery along with systematic lymph node dissection between August 1992 and December 2007 at the National Cancer Center Hospital East. Complete tumor resection was defined as cancer-free surgical margins observed in both gross and histologic examinations. Of these 1,904 patients,

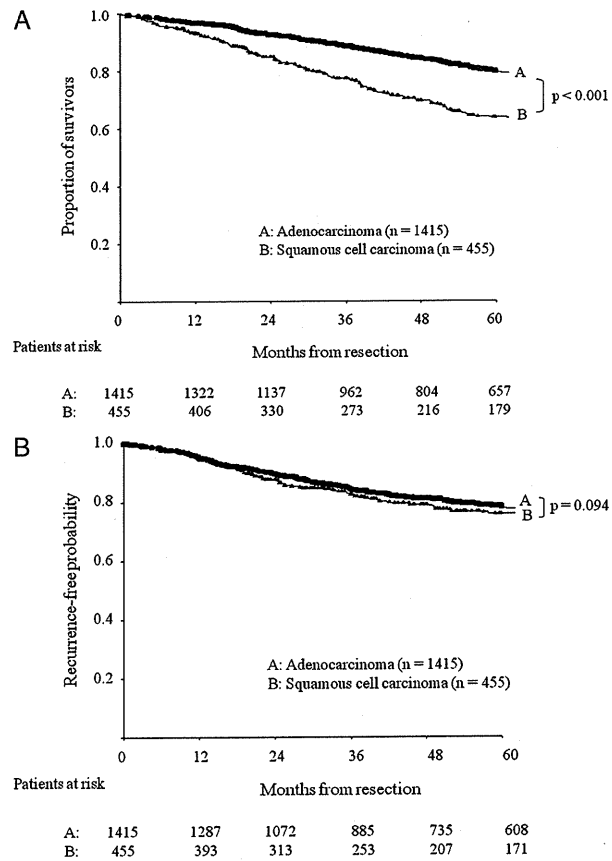

FIGURE 2. Overall survival and recurrence-free probability curves according to histology in the entire cohort. A, Overall survival curves according to histology. B, Recurrence-free probability curves according to histology.

Table 1—*Causes of Death Within 5 Years After Resection for Patients With Adenocarcinoma and SCC*

Characteristics	No. Patients	No. of All-Cause Deaths Within 5 y After Resection (%)	Causes of Death, No. (%)		*P* Value
			Cancer-Related	Other Causes	
Adenocarcinoma	1,415	230 (16)	160 (70)	70 (30)	...
SCC	455	136 (30)	64 (47)	72 (53)	<.001

SCC = squamous cell carcinoma.

34 patients who underwent preoperative chemotherapy, radiation therapy, or both were excluded from this study. The remaining 1,870 patients were included.

Pathologic Evaluation

Disease stages were diagnosed on the basis of the TNM classification of the International Union Against Cancer, seventh edition.[12] Histologic type was determined according to the World Health Organization classification.[13] Blood vessels were identified by hematoxylin-eosin and Victoria blue-van Gieson stainings. Lymphatic ducts were specified by D2-40 staining. Intratumoral vascular invasion (IVI) and lymphatic permeation were histologically diagnosed by identifying cancer cells within blood vessels and lymphatic ducts, respectively. Visceral pleural invasion (VPI) was evaluated with the Victoria blue-van Gieson staining. VPI was defined in the TNM classification.[12] Adenocarcinoma histologic subtypes were categorized into bronchioloalveolar carcinoma (BAC) (nonmucinous or mucinous), papillary, acinar, and solid adenocarcinomas according to the World Health Organization classification.[13] We determined the predominant subtype. One or more tumor nodules with the same histology within the same lobe were defined as intrapulmonary metastasis and classified as T3.[13] Nodules were counted as synchronous primary cancers only if they were of different histologic types.

Patient Follow-up

We examined patients on an outpatient basis at 3-month intervals for the first 2 years and at 6-month intervals thereafter. The follow-up evaluation included physical examination, chest radiography, and blood examination, including pertinent tumor markers. Further evaluations, including CT scan of the chest and abdomen, brain MRI, and bone scintigraphy, were performed when any symptoms or signs of recurrence were observed. Since 2004, integrated PET scan was also performed when indicated.

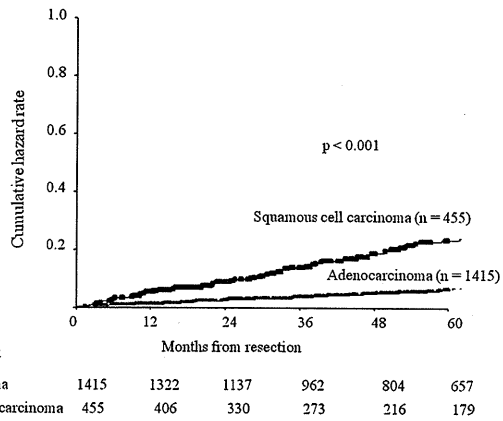

FIGURE 3. Cumulative hazard rate curves of death from other causes according to histology.

We diagnosed recurrence on the basis of physical examination and diagnostic imaging findings and confirmed the diagnosis histologically when clinically feasible. Date of recurrence was defined as the date of histocytologic proof of recurrence. However, in patients diagnosed on the basis of clinicoradiologic findings, date of recurrence was defined as the date of identification of recurrence by a physician. After recurrent diagnosis, surgery, chemotherapy, and radiation therapy individually, and in combination, were performed when clinically feasible.

Clinicopathologic Information

We reviewed each patient's medical record to obtain clinicopathologic information, which included age (dichotomized at the median age of 66), sex, smoking history (never or ever smoker), preoperative serum carcinoembryonic antigen level (cutoff at the normal upper limit of 5 ng/mL), tumor location (upper/middle lobe or lower lobe), tumor laterality (right or left), diameter of the tumor on resected specimens (≤ 2 cm or > 2 cm in stage IA and ≤ 5 cm or > 5 cm in stage II), tumor histology (adenocarcinoma or SCC), lymphatic permeation (presence or absence), IVI (presence or absence), VPI (as defined in the TNM classification[12]; presence or absence), separate tumor nodules in the same lobe of the primary tumor (presence or absence in stage II), number of metastatic N1 lymph nodes (absence, single, or multiple in stage II), and the highest level of involved lymph node station (hilar [10] or interlobar [11], or peripheral [12–14] in stage II), and pathologic stage (stage IA, IB, or II based on the seventh edition TNM classification).[12]

Statistical Analysis

Differences in categorical outcomes were evaluated using the χ^2 test. Continuous variables were compared using the *t* test. To acknowledge the presence of competing risks, we calculated cumulative cause-specific hazard rates with the use of the Kaplan-Meier estimator for each end point.[14,15] The duration of overall survival rate was calculated in months from the date of tumor resection to the date of death due to any cause or the date of the last follow-up. The duration of recurrence-free probability was calculated in months from the date of tumor resection to the date of the first recurrence or the 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 included. In univariate analyses, all cumulative survival curves 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 < 0.05. Analyses were performed using the statistical software SPSS 11.0 (Dr. SPSS II for Windows, Standard Version 11.0; SPSS Inc; Chicago, Illinois) and GraphPad Prism (Prism for Windows, Version 5.02; GraphPad Software, Inc; La Jolla, California). 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.

Table 2—*Patient Characteristics and Histology*

Characteristic	No. Patients (%)	Histology		*P* Value
		Adenocarcinoma	SCC	
Total	1,870	1,415	455	...
Age ± SE, y	1,870	64.2 ± 0.3	68.0 ± 0.4	< .001
Sex				
Women	745 (40)	701 (50)	44 (10)	< .001
Men	1,125 (60)	714 (50)	411 (90)	...
Smoking history				
Never smoker	668 (36)	658 (47)	10 (2)	< .001
Ever smoker	1,202 (64)	757 (53)	445 (98)	...
CEA				
Within normal range	1,249 (67)	993 (70)	256 (56)	< .001
Elevated	618 (33)	420 (30)	198 (43)	...
Not examined	3	...	...	...
Tumor laterality				
Right	1,169 (63)	902 (64)	267 (59)	.058
Left	701 (37)	513 (36)	188 (41)	...
Primary lobe				
Upper or middle lobe	1,197 (64)	952 (67)	245 (54)	< .001
Lower lobe	673 (36)	463 (33)	210 (46)	...
Tumor size ± SE, cm	1,870	2.9 ± 0.1	3.7 ± 0.1	< .001
Lymphatic permeation				
Absent	1,461 (78)	1,110 (78)	351 (77)	.558
Present	409 (22)	305 (22)	104 (23)	...
Intratumoral vascular invasion				
Absent	1,125 (60)	943 (67)	182 (40)	< .001
Present	745 (40)	472 (33)	273 (60)	...
Visceral pleural invasion				
Absent	1,392 (74)	1,064 (75)	328 (72)	.195
Present	478 (26)	351 (25)	127 (28)	...
Intrapulmonary metastasis				
Absent	1,812 (97)	1,368 (97)	444 (98)	.437
Present	58 (3)	47 (3)	11 (2)	...
N status				
N0	1,632 (87)	1,267 (90)	365 (80)	< .001
N1	238 (13)	148 (10)	90 (20)	...
Stage				
IA	914 (49)	777 (55)	137 (30)	< .001[a]
IB	505 (27)	361 (25)	144 (32)	...
II	451 (24)	277 (20)	174 (38)	...

Values given as No. (%) unless otherwise noted. CEA = preoperative serum carcinoembryonic antigen level; normal upper limit at 5 ng/mL. See Table 1 for expansion of the other abbreviation.
[a]Compared with stage IB or higher.

Results

Correlation Between Smoking History and Causes of Death in the Entire Cohort

The median follow-up period was 55 months (range, 1-162 months). Among the 1,870 patients, there were 668 (36%) never smokers and 1,202 (64%) ever smokers. By 5 years after resection, 366 (20%) died. Among 668 never smokers, 55 (8%) died of lung cancer and 35 (5%) died of other causes within the 5 years after resection. In 1,202 ever smokers, there were 169 (14%) deaths related to lung cancer and 107 (9%) from other causes. Figure 1A shows the cumulative hazard functions for all-cause deaths according to smoking history. The cumulative hazard rate of all-cause deaths was significantly different between never smokers and ever smokers ($P < .001$). The cumulative hazard rates of both cancer-related death (Fig 1B) and other causes of death (Fig 1C) for never smokers were significantly lower than those for ever smokers ($P < .001$, respectively).

Overall Survival Rates and Recurrence-Free Probabilities for Patients With SCC and Adenocarcinoma

There were 1,415 (76%) patients with adenocarcinoma and 455 (24%) patients with SCC. Figures 2A and 2B show the overall survival and recurrence-free probability curves of patients in the entire cohort. There was a statistically significant difference in overall survival between adenocarcinoma